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THE ROCKEFELLER INSTITUTE

FOR MEDICAL RESEARCH

REPRINTS

VOLUME XVI

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NEW YORK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
1913

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Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. XI, NO. 4, 1912

ON THE COMBINED ACTION OF MUSCLE PLASMA AND PANCREAS EXTRACT ON SOME MONO- AND DISACCHARIDES.

By P. A. LEVENE and G. M. MEYER.

(*From the Rockefeller Institute for Medical Research, New York.*)

(Received for publication, March 14, 1912.)

Through the experiments reported in a previous communication[1] the present writers have demonstrated that by the combined action of muscle plasma and pancreatic extract *d*-glucose was converted into a disaccharide. On the other hand, under the same conditions of experiment maltose was cleaved into glucose. Naturally it became important to make clear whether the action was applicable also to other sugars. Of the previous writers only Hall[2] allowed the muscle plasma to act on other sugars than glucose. This investigator extended his experiments to *d*-levulose, *l*-arabinose and *d*-xylose, and was led to the conclusion that the glycolytic action of his enzyme mixture was limited to glucose only. In the course of our previous work the optimal conditions for the action of muscle plasma and pancreatic extract were determined with greater certainty, and this made it urgent to repeat and to extend the experiments of Hall; all the more since the action of the enzyme mixture is viewed at present in a different light.

Of the hexoses levulose, mannose and galactose were employed. The mannose was obtained through the courtesy of Dr. Hudson of Washington, and we wish to express our appreciation for his kindness. Of the pentoses *l*-arabinose, *d*-xylose and *d*-ribose were used for the experiments. Lactose was the disaccharide tested.

In regard to hexoses it was found that mannose remained unchanged under the conditions of our experiments, but *d*-levulose showed under the influence of muscle plasma and of pancreatic

[1] Levene and Meyer: this *Journal*, ix, pp. 97–107, 1911.

[2] Hall: *Amer. Journ. of Physiol.*, xviii, pp. 283–294, 1907.

extract a diminution of reducing power which could be restored to nearly the original power by means of hydrolysis with dilute mineral acid.

In this respect our conclusions differ from those of Hall. The analysis of tables published by Hall, however, shows some disappearance of levulose through the action of the enzyme mixture, but the writer is inclined to explain these changes by faults in technique, namely by bacterial contamination. On the other hand, there is no record in his report of the concentration of the sugar employed in his experiments, and the importance of this factor has been emphasized in our previous publication. All our experiments were controlled by bacteriological examination, and only those experiments were taken into consideration which proved free from any bacterial growth. Hence the disagreement between our results and those of Hall in regard to *d*-levulose is probably caused by the difference in the sugar concentration employed in the experiments of Hall and in ours.

Regarding the pentoses our experiments are in full accord with those of Hall, and there was never observed a diminution in the reducing power of the pentose even when the concentration of the sugar solutions employed in the experiments was very considerable.

Also in regard to lactose the results of our experiment harmonize with those of Hall.

On the basis of all this experience one is led to the conclusion that the muscle plasma combined with pancreatic extract possesses the power to cause condensation of only two closely related hexoses, namely of *d*-glucose and of *d*-levulose, and that it remains without action on mannose, xylose, ribose, and lactose. The same enzyme mixture also has the power to bring about the hydrolysis of maltose, but not of lactose.

EXPERIMENTAL PART.

The enzyme mixtures were prepared in the manner described in the previous communication.

All the details of sugar analysis were the same as there described.

The condensed levulose was hydrolyzed by heating on a water bath for two hours with 5 per cent hydrochloric acid.

The results of the analysis are given in the following tables.

d-Levulose

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	LAEVULOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS PER 100 CUBIC CENTIMETERS	PERCENTAGE LOSS
a. At beginning of experiment	0.5	28.7	57.4	20.09		
After thirty-six hours	0.5	25.8	51.6	18.06	2.03	10.1
b. At beginning of experiment	0.5	24.1	48.2	17.2		
After thirty-six hours	0.5	22.4	44.8	16.0	1.2	7.0
After hydrolysis	0.5	23.2	46.4	16.6		
c. At beginning of experiment	1.0	27.9	27.9	10.0		
After thirty-six hours	1.0	26.3	26.3	9.4	0.6	6.0
d. At beginning of experiment	2.0	31.2	15.6	5.46		
After thirty-six hours	2.0	29.4	14.6	5.21	0.25	4.5

Galactose.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	GALACTOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS
a. At beginning of experiment	0.5	23.55	47.1	17.23	
After thirty-six hours	0.5	23.60	47.2	17.28	0
b. At beginning of experiment	0.5	19.0	38.0	13.93	
After thirty-six hours	0.5	19.1	38.2	13.95	0
c. At beginning of experiment	1.0	22.8	22.8	8.34	
After thirty-six hours	1.0	22.6	22.6	8.34	0
d. At beginning of experiment	2.0	22.9	11.45	4.17	
After thirty-six hours	2.0	22.9	11.45	4.17	0

l-Arabinose.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	ARABINOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS
a. At beginning of experiment	0.5	26.4	52.8	14.8	
After thirty-six hours	0.5	26.4	52.8	14.8	0
b. At beginning of experiment	1.0	21.6	21.6	6.37	
After thirty-six hours	1.0	21.6	21.6	6.37	0

d-Xylose.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	XYLOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS
a. At beginning of experiment	0.5	24.1	48.2	12.35	
After thirty-six hours	0.5	24.1	48.2	12.35	0
b. At beginning of experiment	1.0	24.3	24.3	6.22	
After thirty-six hours	1.0	24.3	24.3	6.22	0

Lactose.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	LACTOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS
a. At beginning of experiment	3.0	18.6	6.20	12.0	
After thirty-six hours	3.0	18.5	6.17	12.0	0
b. At beginning of experiment	4.0	17.0	4.25	8.0	
After thirty-six hours	4.0	17.2	4.30	8.0	0

d-Ribose.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	*d*-RIBOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS
At beginning of experiment	0.5	22.2	44.4	15.8	
After thirty-six hours	0.5	22.1	44.2	15.8	0

Mannose.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	MANNOSE, GRAMS PER 100 CUBIC CENTIMETERS	LOSS
a. At beginning of experiment	0.5	24.4	48.8	15.05	
After thirty-six hours	0.5	24.2	48.4	15.05	0
c. At beginning of experiment	1.25	31.8	25.44	7.84	
After thirty-six hours	1.25	31.7	25.4	7.84	0
b. At beginning of experiment	1.0	36.5	36.5	11.25	
After thirty-six hours	1.0	36.5	36.5	11.25	0

In calculating the grams sugar for 100 cc. solution the following values for 1 cc. $\frac{N}{10}$ NH_4CNS equivalent to milligrams sugar were used.

	Mgs.		*Mgs.*
Glucose	3.58	Arabinose	2.95
Laevulose	3.58	Xylose	2.56
Maltose	5.78	Ribose	2.56
Galactose	5.76	Lactose	1.89

Reprinted from The Journal of Biological Chemistry, Vol. XI, No. 4, 1912.

ON THE ACTION OF VARIOUS TISSUES AND TISSUE JUICES ON GLUCOSE.

By P. A. LEVENE and G. M. MEYER.

(*From the Rockefeller Institute for Medical Research, New York.*)

(Received for publication, March 14, 1912.)

The literature on the glycolytic action of various animal organs contains most contradictory and confusing statements. While some writers claimed the presence of sugar-destroying enzymes in all organs and tissues, other observers detected such enzymes only in few organs, and only under very definite conditions, namely, in the presence of some auxiliary substance. Thus, in recent years, Arnheim and Rosenbaum[1] and Stoklasa[2] and his co-workers claimed a general distribution of sugar-destroying enzymes in all animal tissues. Rapoport[3] observed gylcolytic action only in blood and fibrin and obtained negative results from his experiments with other organs. Finally, R. Hirsch[4] and O. Cohnheim[5] observed that the glycolytic action is brought about by the combined action of pancreas extract and of the liver, or by pancreas extract and muscle plasma.

It is possible that the observations reported by every one of the writers are correct, and the apparent contradictions were brought about by the different conditions of the respective experiments. It became evident from our work on the combined action of muscle plasma and pancreatic extract that alone variations in the sugar concentration may change the results of the experiments to such an extent that a marked disappearance of glucose will

[1] *Zeitschr. f. physiol. Chem.*, xl, p. 220, 1903–1904.

[2] *Pflüger's Archiv*, ci, p. 311, 1904.

[3] *Zeitschr. f. klin. Med.*, lvii, p. 208, 1905.

[4] *Hofmeister's Beiträge*, iv, p. 535, 1903.

[5] *Zeitschr. f. physiol. Chem.*, xxxix, p. 336, 1903; xlii, p. 401, 1904; xliii, p. 547, 1904–1905; xlvii, p. 253, 1906.

take place in one instance, and no change in the sugar content in the other. An analogous observation was made in the work on leucocytes, which will be reported subsequently.

Another factor determining the result of the experiment lies in the degree of alkalinity or acidity of the solutions used in the experiment. The importance of this factor was pointed out first by the work of Hall, and was corroborated by our own experiments. In agreement with Hall we found that Henderson's phosphate mixture offers the best medium for the study of the so-called glycolytic process. Still another cause for divergence in the characteristics of the results one may find in the difference in the species of the animal whose organs were employed in the experiment. Recent work on the enzymes of animal tissues has brought to light the great differences in the enzyme content of analogous organs of animals belonging to different species. Of course some of the discrepancies in the results of individual writers may have been caused by the different degrees of antiseptic precaution exercised by them. Only rarely were experiments controlled by bacteriological examinations. Further, the analytical methods employed by different workers varied greatly in their accuracy. And yet another source for possible error may be found in the fact that rarely was there made an attempt to search for the products of the disappearing sugar.

The knowledge of the products formed in course of the experiment is important not only for theoretical reasons but as a means of detecting bacterial contamination. Thus in all our experiments of the last two years carbon dioxide was only rarely detected in the reaction mixture, and its presence always indicated bacterial contamination; therefore, we are inclined to believe that in the experiments of other writers, where there was reported the formation of carbon dioxide from sugar, this resulted from bacterial activity, and not through the actions of tissue enzymes.

All these considerations led us to subject to a revision all the older observations on the presence in animal organs of either "glycolytic" enzymes or of activators of the enzymes, all the more since it became evident that the so-called glycolysis under the combined action of muscle plasma and pancreas consisted in a condensation and not in a destruction of glucose. In course of this

work the organs of the rabbit and of the dog were employed. In one series of experiments the enzymotic effect of the tissue pulp was tested and in another of the tissue juices. A still different series of experiments aimed to investigate the presence in various organs of enzyme activators. For this purpose the action of various organs aided by extracts of other organs was tested. Every experiment was controlled by bacteriological examinations, made by Dr. Bronfenbrenner; only experiments that proved free from any bacterial growth were taken into consideration.

The results of the experiments were as follows:

A. In the experiments with the rabbit, without the aid of activators only the action of liver and of muscle tissues were tested. The results in both experiments were negative. With the aid of pancreas extract also only the same two tissues were tested. Positive results were obtained only with the muscle plasma.

B. In the experiments with the dog without the aid of activators the following tissue juices were employed: muscle, lung, intestine, kidney, pancreas and spleen. All experiments were negative.

As activators the extracts of the pancreas and of the spleen were employed. With each activator were employed the same tissue juices as in the first series. The results were the following. The addition of pancreas extract did not alter the action of the tissue juices; on the other hand, after the addition of spleen extract as activator there was observed a fall of the reducing power of the sugar solution in the experiments with muscle, lung, liver and pancreas. The action was of very moderate intensity.

In the experiments with tissue pulp the following organs were used: muscle, spleen, liver, lung and pancreas. The results in all experiments were negative, excepting the liver. The experiments with liver tissue and additional glucose showed at the end of the experiments no change in the original reducing power; on the other hand, in the control experiments with liver tissue alone there was observed an increase in the reducing power. Hence it follows that in the experiment with additional glucose there was a compensation of phenomena so that the rise of glucose formation was observed by a simultaneous disappearance of glucose.

With the addition of pancreas extract as activator the same organs were used. The results were the same as in previous series.

With the addition of spleen extract as activator the presence of enzymotic action was observed in the experiments with muscle, lung, liver and pancreas tissues.

Thus in the dog the spleen and not pancreas is the organ containing the activator for the enzyme causing the condensation of glucose.

The general conclusion from the present experiments is that under the conditions here reported, namely, in the presence of antiseptics and under the conditions where access of oxygen is not totally excluded, animal tissues or their juices, aided or unaided by auxiliary substance fail to bring about a destruction of glucose. Wherever a fall in the reducing power of a sugar solution was brought about by the combined action of tissue and activator this was due to a condensation of the glucose. However, the writers realize that under some other conditions an actual glycolysis may take place and it is hoped the exact conditions will be determined at some future date.

EXPERIMENTAL PART.

The animals used in the experiments were killed by bleeding from the jugular vein. The organs were removed under aseptic conditions and immediately employed for the preparation of either plasma or tissue pulp.

Tissue Plasma. For the preparation of this, the organs were hashed and extracted for several hours with the Henderson's phosphate mixture, and then strained through cheese cloth; the residue was ground with sand and pressed in a Buchner press at 300 atmospheres. All the liquids were combined and employed for the experiments.

Tissue Pulp. Five grams of the freshly prepared organ pulp were added to a flask containing 45 cc. of a solution of glucose in Henderson's phosphate solution.

Intestinal Extract was prepared in the following way. The intestines were washed and the mucous membrane scraped off with a knife. The substance was taken up in a Henderson's phosphate solution containing glucose and well agitated.

Activators. Ten grams of the hashed organ were treated in identically the same manner as previously described for the pancreas.

The final solution was made up to 10 cc. and 1 cc. added to each flask of 50 cc.

Sugar Estimations. The reducing power was determined in all cases on the strained mixture. Ten cubic centimeters of this liquid were coagulated by boiling and the addition of acetic acid; and made up to 100 cc. without filtering. This solution was filtered through a dry filter and five, ten or more cubic centimeters used for each reduction. The reduced copper was determined by the Volhard method.

Organs of a rabbit.

A rabbit was starved for three days and placed in a room below freezing temperature for three hours prior to its execution.

	TISSUE	ACTIVATOR	NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	GLUCOSE GRAMS PER 100 CUBIC CENTIMETERS	LOSS PER CENT
			cc.			
Before	Muscle	None	20.0	40.0	15.32	
After			20.0	40.0		
Before	Muscle	Pancreas	24.0	48.0	17.18	
After			21.0	42.0	15.03	12.5
Hydrolyzed			23.6	47.2	16.90	
Before	Liver	None	23.5	47.0	16.82	
After			23.5	47.0		
Before	Liver	Pancreas	22.9	45.8	16.39	
After			23.1	46.2	16.53	

1 cc. liver plasma—no reduction.
1 cc. liver plasma hydrolyzed—no reduction.

Tissue plasma of dog.

	TISSUE	ACTIVATOR	NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	GLUCOSE GRAMS PER 100 CUBIC CENTIMETERS	LOSS PER 100 CUBIC CENTIMETERS	LOSS PER CENT
			cc.				
Before	Muscle	None	20.6	41.2	14.55		
After			20.6				
Before	Muscle	Spleen	23.0	46.0	16.5		
After			21.9	43.8	15.6	0.9	4.8
Hydrolyzed			22.3	44.6	16.0		
Before	Muscle	Pancreas	21.6	43.2	15.5		
After			21.5	43.0			
Before	Muscle	Spleen	27.0	54.0	19.3		
After			25.2	50.4	18.06	1.24	6.4
Before	Muscle	Pancreas	30.0	60.0	21.5		
After			30.0	60.0	21.5		
Before	Lung	None	22.0	44.0	15.75		
After			21.7	43.4	15.55		
Before	Lung	Spleen	20.1	40.2	14.3		
After			16.9	33.8	12.05	2.25	15.7
Hydrolyzed			19.0	38.0	13.65		
Before	Lung	Pancreas	20.1	40.2	14.4		
After			20.0	40.0			
Before	Lung	Spleen	28.5	57.0	20.4		
After			27.5	55.0	19.69	0.71	3.5
Before	Intestine	None	22.5	45.0	16.08		
After			22.8	45.6	16.30		
Before	Intestine	Spleen	20.6	41.2	14.75		
After			20.8	41.6	14.89		
Before	Intestine	Pancreas	20.4	40.8	14.60		
After			20.4	40.8			
Before	Intestine	Pancreas	33.0	66.0	23.62		
After			33.1	66.2			
Before	Intestine	Spleen	32.6	65.2	23.32		
After			32.8	65.6	23.48		
Before	Kidney	Spleen	26.6	53.2	19.04		
After			26.8	53.6	19.18		
Before	Pancreas	Spleen	29.6	59.2	21.19		
After			29.5	59.0			
Before	Spleen	Pancreas	32.3	64.6	23.12		
After			32.2	64.4			

Organ pulp of dog, without activator.

	TISSUE	NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	GLUCOSE GRAMS PER 100 CUBIC CENTIMETERS	LOSS PER 100 CUBIC CENTIMETERS	LOSS PER CENT
		cc.				
Before.....	Muscle	16.9	33.8	12.10		
After.....		17.0	34.0			
Before.....	Spleen	23.7	47.4	16.96		
After.....		23.7	47.4			
Before.....	Liver	19.2	38.4	13.74		
After.....		19.0	38.0	13.60	0.14	1.0
Before.....	Pancreas	25.4	50.8	18.18		
After.....		25.5	51.0			
Before.....	Lung	23.7	47.4	16.96		
After.....		23.6	47.2			

Organ pulp of dog, with activators.

	TISSUE	ACTIVATOR	NH_4CNS PER CUBIC CENTIMETER	GLUCOSE GRAMS PER 100 CUBIC CENTIMETERS	LOSS PER 100 CUBIC CENTIMETERS	LOSS PER CENT
Before...	Muscle	Spleen	40.4	14.46		
After....			39.2	14.03	0.43	2.9
Before...	Lung	Spleen	34.4	12.31		
After....			33.8	12.10	0.21	1.7
Before...	Liver	Pancreas	35.4	12.70		
After....			35.4	12.70	0.00	
Before...	Liver	Spleen	34.2	12.24		
After....			33.8	12.10	0.14	1.1
Before...	Pancreas	Spleen	32.0	11.45		
After...			31.0	11.09	0.36	3.1

Organ pulp of dog, without glucose.

5 grams muscle pulp ..... No reduction
5 grams spleen pulp ..... No reduction
5 grams pancreas pulp ..... No reduction
5 grams lung pulp ..... No reduction
5 grams liver pulp { Before..... $10 - 9.3 - 0.7$ $NH_4CNS = 0.25$ per cent
Hydrolyzed $10 - 7.9 - 2.1$ $NH_4CNS = 0.75$ per cent

Reprinted from The Journal of Biological Chemistry, Vol. XI, No. 4, 1912

THE ACTION OF LEUCOCYTES ON GLUCOSE.

By P. A. LEVENE and G. M. MEYER.

(*From the Rockefeller Institute for Medical Research, New York.*)

(Received for publication, March 14, 1912.)

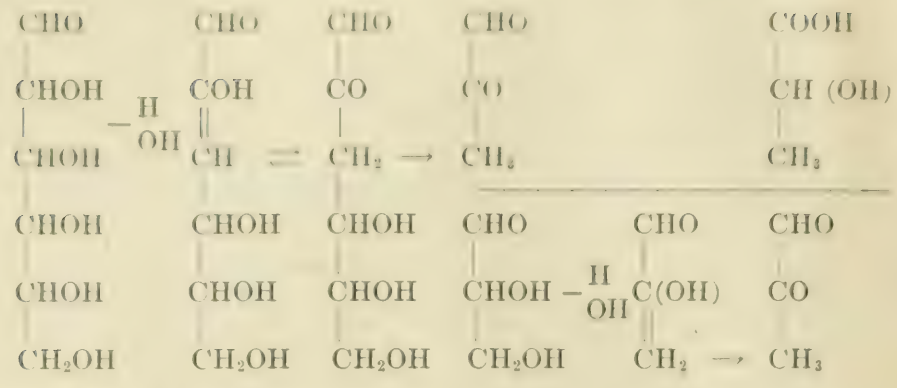
The final products of sugar combustion in the animal organism are carbonic acid and water. This information is undisputable. The knowledge of every other phase in the complex process of glycolysis has not advanced beyond the state of conjecture. The paucity of undisputed knowledge regarding this very important biological process is due to the fact that intravital reactions are made visible only with great difficulty, and secondly to the great variety of theoretically possible processes which may lead to the breaking of the links between the individual carbon atoms of the sugar molecule.

There are three principal types of sugar degradations. One begins directly with the oxidation of the end carbon atoms, the second consists in a gradual dissociation of formaldehyde. It represents a reversion of the synthesis of sugar from formaldehyde. The first step of this reaction leads to the formation of pentose from glucose. The reaction of the third type leads to a splitting of the six-carbon chains of glucose into two three-carbon chains—with the formation of either dioxyacetone or of lactic acid as the first phase of the reaction.

$$\text{I.}\quad \begin{matrix}\mathrm{CH_2OH}\\ |\\ (\mathrm{CHOH})_4\\ |\\ \mathrm{CHO}\end{matrix} \rightarrow \begin{matrix}\mathrm{CH_2OH}\\ |\\ (\mathrm{CHOH})_4\\ |\\ \mathrm{COOH}\end{matrix} \rightarrow \begin{matrix}\mathrm{COOH}\\ |\\ (\mathrm{CHOH})_4\\ \\ \mathrm{COOH}\end{matrix} \rightarrow \begin{matrix}\mathrm{CO_2}\\ +\\ 2(\mathrm{COOH})_2\\ +\\ \mathrm{CO_2}\end{matrix}$$

$$\text{II.}\quad \mathrm{CH_2OH(CHOH)_3CHOH\ CHO} \rightleftharpoons \mathrm{CH_2OH(CHOH)_3CHO} + \mathrm{HCHO}$$

III*a*.



b. $C_6H_{12}O_6 = 2\ CH_3CHOH-COOH$

c. $C_6H_{12}O_6 = 2\ CH_2OH-CO-CH_2OH$

Each one of the three types of reactions was taken into consideration for the explanation of the process of sugar combustion in the body and attempts were made to interpret the process on the basis of every one of the three reactions.

The method employed for the purpose of bringing to light the mechanism of biological cleavage of sugar were the following:

1. The search in the tissues for substances that may originate from carbohydrates.

2. Experiments on the action of tissues and tissue extracts on sugar.

3. Perfusion of organs with carbohydrates and their cleavage products.

4. Feeding healthy and diabetic animals on carbohydrates and their oxidation product.

None of the methods has furnished thus far convincing evidence which would permit the acceptance, or would force the rejection of either one of the possible interpretations of sugar combustion in the animal. For the reasons which will become evident later, we shall briefly review the work which aimed to analyze the possibility of the intermediate cleavage of hexose into two molecules each containing a chain of three carbons. Four substances had been named in connection with the biological sugar combustion: lactic acid, glyceric aldehyde, methylglyoxal and dioxyacetone.

In connection with sugar combustion in the animal organism only lactic acid received serious consideration. The formation of lactic acid in surviving tissues at the expense of disappearing glycogen was accepted by older writers. In those experiments the

influence of bacteria was not excluded, and the subject received a revision in recent years The views of the writers who were actively engaged in the investigation of lactic acid in the animal organism are equally divided, some regarding protein and others carbohydrates as the source of the substance. The writers who in recent years contributed evidence in support of the carbohydrate origin of lactic acid were Spiro,[1] Hoppe-Seyler[2] and his co-workers, Araki[3] and Zillessen,[4] Embden,[5] and particularly Graham Lusk and A. R. Mandel.[6] The most emphatic partisan of the protein origin of lactic acid was Minkowski[7] and his views are supported by evidence adduced by Asher and Jackson,[8] and by Neuberg and Langstein.[9]

The views of Hoppe-Seyler and his co-workers were based on observations on animals placed in conditions which brought about insufficient oxygenation of the tissues. In all such conditions there was observed the elimination of lactic acid through the urine. Inouye and Saiki made similar observations in epilepsy. Embden reached his conclusions on the basis of perfusion experiments. The perfusion of livers rich in glycogen resulted in lactic acid formation. The same result was observed when blood containing sugar was perfused through a liver poor in glycogen. On the other hand, perfusion of a liver freed from glycogen with blood of a very low sugar content failed to bring about lactic acid formation. Lusk and Mandel based their view on experiments on dogs which received combined phloridzin and phosphorus treatment. It is known that phosphorus injected into normal animals causes the elimination of lactic acid. On the other hand, the injection of phloridzin caused the removal, through the urine, of glucose from the body tissues. If the phosphorus injection was preceded by a phloridzin injection it failed to give rise to an elimination of lactic acid. The

[1] *Zeitschr. f. physiol. Chem.*, i, p. 111, 1877.

[2] *Festschr. zu Virchow's Jubiläum.*

[3] *Zeitschr. f. physiol. Chem.*, xv, pp. 335 and 546, 1891; xvi, pp. 201 and 453, 1892; xvii, p. 311, 1893; xix, p. 422, 1894.

[4] *Zeitschr. f. physiol. Chem.*, xv, p. 387, 1891.

[5] *Centralblatt f. Physiol.*, xviii, p. 832, 1905.

[6] *Amer. Journ. of Physiol.*, xvi, p. 129, 1906.

[7] *Arch. f. exp. Path. u. Pharm.*, xxi, p. 67, 1886; xxxi, p. 214, 1893.

[8] *Zeitschr. f. Biol.*, xli, p. 393, 1901.

[9] *Arch. f. Physiol.*, Suppl. 1903, p. 514.

interpretation given by Lusk and Mandel to these observations was that normally under the influence of phosphorus lactic acid is formed at the expense of glucose; and that phloridzin in removing glucose also removes the mother substance of lactic acid.

On the other hand, Minkowski observed that birds, after removal of the liver, eliminate through their urine considerable quantities of lactic acid, and that its value is influenced by the protein intake and not by that of carbohydrates. Jackson and Asher in perfusion experiments failed to detect any influence of carbohydrate on the lactic acid formation and Neuberg and Langstein demonstrated the appearance of lactic acid in the urine after administration of alanine. Thus the evidence in support of either view was mostly indirect and not sufficiently convincing. These considerations impelled us a year ago to undertake the study of the products formed in course of glycolysis by means of tissue extracts. The results were all negative. For various reasons it was deemed advisable to test the influence on glucose of living leucocytes. Lepine[10] and his co-workers and Mayer[11] had already advanced the view that leucocytes were concerned in the process of glycolysis. Their evidence was indirect and the products of leucocytic glycolysis remained unexplained by them.

In the present investigation all the experiments were performed under absolutely aseptic conditions. The solutions were always tested for aërobic and anaërobic microörganisms by Dr. Bronfenbrenner to whom we are greatly indebted for that part of the work. The leucocytes were suspended in a sugar solution containing 15 per cent of the Henderson phosphate mixture. The results of the experiments were the following:

1. Under the influence of leucocytes a sugar solution loses part of its reducing power.

2. The reducing power cannot be restored to the original by boiling with mineral acids.

3. The rate of glycolysis is in inverse proportion to the sugar concentration. (The last two points are interesting in connection with the influence of muscle plasma and pancreatic extract of glucose. There the fall in the reducing power was in direct pro-

[10] *Le diabète sucré*, Paris, 1909.

[11] *Arch. de Physiol.* 2.

portion to the sugar concentration, and the original reducing power could be restored by hydrolysis with mineral acids.)

4. If distilled water is employed in place of the phosphate mixture the leucocytes fail to exert any influence on glucose.

5. If toluol is added to the phosphate mixture the leucocytes do not demonstrate any action on glucose.

6. As product of the action of leucocytes on glucose, paralactic acid was discovered. It was identified as the zinc salt. Volatile acids were not detected.

7. The quantity of lactic acid found was lower than that of the disappeared glucose. Whether the missing sugar underwent decomposition into other substances than lactic acid, or was used for synthetic purposes by the leucocytes remains to be established.

EXPERIMENTAL.

Leucocytes. Medium sized dogs were given two injections of 1.5 cc. turpentine into the pleural cavity at an interval of three days. Ether narcosis was used at the first injection. Eighteen hours after the second injection the liquid which had formed in the pleural cavity was withdrawn by aspiration. This exudate, which contained the greater portion of the turpentine, was discarded. The following day the aspiration was repeated. This fluid was received into sterile bottles. The color of the exudate was a straw yellow occasionally tinged red. The quantity of exudate obtained from each dog varied greatly. Three or more dogs were injected simultaneously so as to insure an ample supply of leucocytes. The combined exudate was centrifugalized and the leucocytes washed twice with sterile physiological saline. The centrifugal flasks contained glass beads to facilitate the breaking up of the agglutinated mass and thus aid proper washing and mixing.

Solutions. In the first three experiments the leucocytes were well shaken with sterilized 1 per cent Henderson phosphate solution and this suspension added to flasks containing the desired quantity of glucose. Merck's "highest purity" glucose was used in all experiments. Just enough water was added previous to sterilizing, so that it remained liquid after cooling. In later experiments, where only one concentration of glucose was desired,

the sterilized glucose syrup was dissolved in the phosphate solution and this then added to the leucocytes and well shaken. The quantity of glucose-phosphate solution which was added to the leucocytes depended to a certain extent upon the quantity of leucocytes at hand, although no attempt was made to count the cells. The mixture was usually made up to a volume nearly one-half of that of the total exudate. As the exudate contains approximately 10 per cent leucocytes, the glucose was acted upon by a 20 per cent leucocyte suspension. The mixture was kept for thirty-six hours at 37°C. The flasks were stoppered with cotton and well covered and sealed with tin foil to prevent evaporation.

Methods of Analysis. Immediately after mixing the leucocytes with glucose and after thirty-six hours samples were withdrawn for analysis. The leucocytes were allowed to settle and the clear supernatant liquid only used for the sugar determinations. The liquid was freed of protein by boiling and acetic acid. The reduced copper was estimated by the Volhard method. The details of the sugar determination are identical with those already described in a previous communication.

Carbon Dioxide. A measured volume of the leucocyte mixture was used for this determination according to the method of Fresenius and Classen. The liquid was freed as far as possible from protein by heat while still alkaline. Phosphoric acid was used to acidify the mixture.

Lactic Acid. The filtered residue from the carbon dioxide determination was evaporated nearly to dryness. Anhydrous sodium sulphate was then added and all carefully ground in a mortar to an impalpable dry powder. This was then extracted with anhydrous ether, until the extract gave no further test for lactic acid. All extracts were combined and freed of ether. The residue was taken up in a little water and boiled with zinc carbonate. The filtered aqueous solution was evaporated to dryness and the total weight obtained. This residue was usually more or less colored. It was redissolved in water and clarified by boiling with animal charcoal. After evaporating to a small volume zinc lactate soon crystallized. The crystals were used for further analysis and identification.

Volatile Acid. The uncoagulated leucocyte mixture was distilled with steam into $\frac{N}{10}$ barium hydrate. Glacial phosphoric acid was added through a separatory funnel in such a manner as

to prevent any loss of CO_2 and volatile acids. The adapter from the condenser dipped into the barium hydrate which was contained in a tall stoppered cylinder, connected with a series of wash bottle likewise containing barium hydrate and finally a soda lime tube to guard against absorption of carbon-dioxide through accidental back pressure.

A. *Experiments showing the relation between rate of glycolysis and concentration of sugar solution.*

Experiment I. 600 cc. exudate was obtained from two dogs. Four flasks with glucose at different concentrations were prepared.

	Cubic centimeters used	Cubic centimeters NH_4CNS	NH_4CNS per cubic centimeter	Per cent of glucose	Loss per 100 cubic centimeters	Percentage loss
a. At beginning of experiment.....	0.5	19.0	38.0	13.6		
After thirty-six hours.....	0.5	19.0	38.0	13.6	0	0
b. At beginning of experiment	1.0	30.0	30.0	10.74		
After thirty-six hours.....	1.0	30.0	30.0	10.74	0	0
. At beginning of experiment.....	2.0	45.0	22.5	8.05		
After thirty-six hours.....	2.0	39.5	19.7	7.05	1.00	12.4
After hydrolysis.....	2.0	39.5	19.7	7.05	0	0
. At beginning of experiment.....	2.0	36.6	18.0	6.4		
After thirty-six hours.....	2.0	33.0	16.5	5.9	0.5	7.8
After hydrolysis.....	2.0	33.0	16.5	0	0	0

Experiment II. 500 cc. exudate. Four flasks at two different concentrations. The remainder 100 cc. leucocyte suspension used as control.

	Cubic centimeters used	Cubic centimeters NH_4CNS	NH_4CNS per cubic centimeter	Glucose per 100 cubic centimeters	Loss	Percentage loss
I. At beginning of experiment....	1.0	24.4	24.4	8.72		
After thirty-six hours.....	1.0	22.5	22.5	8.07	0.65	7.5
II. At beginning of experiment....	1.0	23.9	23.9	8.55		
After thirty-six hours.....	1.0	22.0	22.0	7.87	0.68	7.9
III. At beginning of experiment....	1.0	18.5	18.5	6.62		
After thirty-six hours.....	1.0	16.8	16.8	5.91	0.71	10.7
IV. At beginning of experiment....	1.0	18.3	18.3	6.55		
After thirty-six hours.....	1.0	16.5	16.5	5.90	0.65	10.0

B. *Experiments aimed to test the formation of acid during the process of glycolysis.*

Experiment Ia. 100 cc. control leucocytes from A experiment II acidified and distilled with steam into $\frac{N}{10}$ $Ba(OH)_2$. The $Ba(OH)_2$ was filtered from the carbonate and titrated; phenolphthalein used as indicator.

20 cc. $\frac{N}{10}$ $Ba(OH)_2$ required 17.6 cc. $\frac{N}{10}$ HCl = 2.4 cc.

Experiment Ib. 99 cc. of the leucocyte-glucose mixture was treated in identically the same manner. The 99 cc. consisted of—

	SUGAR BEFORE	SUGAR AFTER	LOSS
	grams	*grams*	*grams*
I. 30 cc....	2.61	2.42	0.19
II. 27 cc....	2.30	2.12	0.18
III. 25 cc....	1.65	1.47	0.18
IV. 17 cc....	1.13	1.00	0.13
	7.69	7.01	0.68

37.3 cc. $\frac{N}{10}$ $Ba(OH)_2$ after distillation and filtering from the carbonate required 34.7 cc. $\frac{N}{10}$ acid = 2.6 cc. $\frac{N}{10}$ neutralized.

C. *Experiments showing the development of lactic acid in course of glycolysis.*

Experiment Ia. The residue from B. Experiment Ia. was extracted in a Schwartz extractor with ether for lactic acid. There was only the merest trace of residue obtained from the ether extract, which was neutral to litmus.

Experiment Ib. The residue from B. Experiment Ib. was extracted for lactic acid. Yield of crude zinc lactate dried at 100° = 0.3184 gram.

0.0961 gram recrystallized salt, air dried after drying to constant weight lost 0.0106 gram H_2O = 12.3 per cent H_2O.

Calculated for two molecules H_2O = 12.5 per cent.

0.1362 gram recrystallized salt on ignition yielded 0.0458 ZnO = 33.62 per cent.

Calculated = 33.4 per cent.

Experiment II. Contents of flasks I and II (A. Experiment II) were coagulated and extracted for lactic acid. No lactic acid was obtained from II. The yield from I = 0.305 gram crude zinc lactate.

0.2616 gram recrystallized and air dried salt on heating to constant weight at 100° lost 0.032 gram = 12.35 per cent H_2O.

Calculated for two molecules H_2O = 12.5 per cent.

0.1135 recrystallized and dried at 110° after ignition gave 0.0383 ZnO = 33.7 per cent ZnO.

Calculated = 33.4 per cent.

Experiment IIIa. Contents of flask I Experiment E (170 cc.) was coagulated and filtered, the filtrate neutralized and evaporated nearly to dryness. The syrup was acidified with a small quantity of glacial phosphoric acid and

well ground with highest purity anhydrous sodium sulphate. This powder was then repeatedly extracted with hot ether. The ether extracts were combined and treated as previously mentioned to obtain zinc lactate. Yield of crude zinc lactate dried = 0.4798 gram.

0.2868 gram recrystallized at 100° dried salt dissolved in 2.84 cc. of water gave a rotation in the polariscope of — 0.22°.

0.3274 gram of the recrystallized salt after drying at 110° weighed 0.2868 gram.

Loss = 0.0406 gram = 12.31 per cent H_2O.

Calculated for two molecules H_2O = 12.5 per cent.

0.0999 gram of dried zinc salt gave 0.0337 gram ZnO = 33.8 per cent ZnO.

Calculated = 33.4 per cent.

Experiment IIIb. The controls, flasks II and III Experiment E (170 cc.) were subjected separately to the identical treatment as flask I. No zinc lactate was obtained.

D. *Experiments showing effect of distilled water and dilution; 700 cc. exudate.*

I. Three flasks with 1.86 grams glucose, mixed with leucocytes and phosphate solution.

II. Two flasks with 3.75 grams glucose with leucocytes and distilled water.

III. 50 cc. leucocytes and phosphate solution set aside for control.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	GLUCOSE PER 100 CUBIC CENTIMETERS	LOSS	PERCENTAGE LOSS
I. At beginning of experiment....	4.0	35.0	8.75	3.12		
After thirty-six hours.....	4.0	32.4	8.1	2.90	0.22	7.8
At beginning of experiment....	4.0	32.8	8.2	2.94		
After thirty-six hours.....	4.0	30.0	7.5	2.68	0.26	8.8
At beginning of experiment....	4.0	32.6	8.15	2.92		
After thirty-six hours.....	4.0	29.6	7.40	2.65	0.27	9.2
II. At beginning of experiment....	2.0	39.3	19.65	7.04		
After thirty-six hours.....	2.0	39.4	19.70	7.05	0	0
At beginning of experiment....	2.0	35.4	17.7	6.32		
After thirty-six hours.....	2.0	35.6	17.8	6.33	0	0
III. 25 cc. of leucocytes and phosphate solution gave no appreciable reduction of Fehling's solution. There was likewise no reduction after two hours hydrolysis with 2 per cent HCl.						

E. *Effect of adding toluol; control of action of phosphate solution on glucose; 800 cc. exudate.*

I. One flask of 200 cc. glucose, leucocytes and phosphate solution.
II. One flask of 200 cc. as I, with addition of toluol.
III. Glucose and phosphate solution.

	CUBIC CENTIMETERS USED	CUBIC CENTIMETERS NH_4CNS	NH_4CNS PER CUBIC CENTIMETER	GLUCOSE PER 100 CUBIC CENTIMETERS	LOSS	PERCENTAGE LOSS
I. At beginning of experiment.....	2.0	34.0	17.0	6.05		
After thirty-six hours.....	2.0	31.5	15.75	5.60	0.45	7.4
II. At beginning of experiment.....	2.0	34.3	17.15	6.1		
After thirty-six hours.....	2.0	34.3	17.15	6.1	0	0
III. At beginning of experiment.....	2.0	35.2	17.6	6.3		
After thirty-six hours.....	2.0	35.2	17.6	6.3	0	0

Reprinted from The Journal of Biological Chemistry, Vol. XI, No. 4, 1912

ON THE ACTION OF TISSUE EXTRACTS CONTAINING NUCLEOSIDASE ON α AND β METHYLPENTOSIDES.

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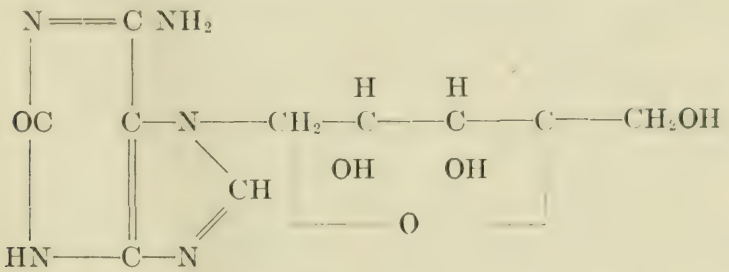
(Received for publication, March 14, 1912.)

Through the work of Levene and Jacobs it was established that the purine bases enter the molecule of the plant nucleic acid and of some animal nucleic acids in the form of a *d*-riboside. The structure of these may be represented by that of guanosine:



Regarding two points of their structure there exists at present no definite information. The first concerns the place of the union between the two molecules. The formula given here assumes a union in position 7 of the purine base, but the experimental evidence admits with the same degree of probability also the position 8. The second pertains to the two possible stereoisomeric forms of the pentosides. The assumption of the lactonic structure of glycosides admits of the existence of two isomeric forms of each glycoside conditioned by the asymmetric nature of the end-carbon. Hence theoretically there are possible α and β forms of the nucleosides in the same manner as there exist α and β forms of any other pentoside, and it therefore remains to be established whether the natural nucleosides belong to the α or the β series.

Three methods are available for the solution of the last problem. The first was introduced by Fischer[1] and is based on the specific

[1] *Zeitschr. f. physiol. Chem.*, xxvi, p. 61, 1898.

power of certain enzymes to cause the cleavage of glycosides of one order leaving intact the other stereoisomeric form. Thus emulsin is capable of hydrolyzing β glycosides but not the α forms. On the contrary maltose has no capacity for disrupting the β forms, but possesses one for the α forms. This method was not available in the present investigation, for the reason that neither emulsin nor maltose, in the form as we were able to procure them, had the capacity for cleaving the nucleosides.

The second method was introduced by Armstrong[2] and is based on the observation of the mutarotation of the sugar liberated from the glycoside. If the mutarotation is analogous to the rotation which characterizes the transformation of the α-isomer into the stable form, the glycoside is regarded as the α-glycoside and *vice versa*. This method is available when there exists an enzyme capable of hydrolyzing the glycoside with a sufficiently high degree of intensity, and besides, when the transformation of the isomeric sugars into their stable forms proceeds at a low rate of velocity. Unfortunately the sugar liberated from the nucleosides possesses a low rotation and is very rapidly transformed into the stable form, so that it shows only a low degree of mutarotation, which becomes evident only under very definite conditions. On the other hand, the cleavage of the nucleosides by the nucleases proceeds very slowly, so that in a moderately short interval only little ribose is liberated. Another difficulty was encountered in the fact that the hypoxanthine formed in course of the cleavage (inosin was used in the experiments) combined with some of the unchanged nucleoside, giving rise to a precipitate of carnin. All these difficulties made the method of Armstrong of little value for the purposes of the present investigation.

The third method was introduced by Hudson,[3] and is based on certain numerical values of the rotations of sugars and their glycosides. This method permits of establishing the nature of a glycoside when only one form is known, but when simultaneously there exists information regarding the specific rotation of at least one form of the sugar. According to Hudson the difference in the molecular specific rotation of two sugars has a constant value of

[2] *Journ. Chem. Soc.*, lxxxiii, p. 1305, 1903.

[3] *Journ. Amer. Chem. Soc.*, xxxi, p. 66, 1909.

16200. Hence the knowledge of the value of the specific rotation of one form permits of obtaining the value of the other form. Further, the sum of the molecular specific rotations of the two forms remains constant for every sugar and all its glycosides. Thus, if one possesses the knowledge of the sum of the specific rotations of the two forms of a sugar, he is also in possession of the information regarding the sum of the specific rotations of the glycosides. Hence it is possible to calculate by a simple arithmetical process the specific rotation for the second isomeric glycoside when that of the first is known.

Thus accepting the difference in specific molecular rotation expressed by the formula $-\alpha+\beta=\frac{1620}{150}=108$, and accepting for one isomer $[\alpha]_D=-14.65$, the rotation of the other will equal $-\beta=-\alpha-108=-122.65°$. When the rotations of the two forms are given Hudson suggested the following rule for naming the α and β forms: "The names should be selected that for all sugars which are genetically related to *d*-glucose the subtraction of the rotation of the β-form from the α-form gives a positive difference and for all sugars which are genetically related to *l*-glucose an equal negative difference." According to this rule the unknown form of *d*-ribose is to be named the β-form.

The information obtained in this manner furnishes also the value for the sum of the rotations of the two isomers of *d*-ribose, $-137.30°$, which is also the sum of the rotations of all glycosides of the same sugars. Applying this rule for inosin, of which the known form has the rotation of $-49.2°$, one is led to the conclusion that the other form has the rotation of $-87.80°$, and is therefore β-*d*-riboside.

Thus this process of reasoning leads to the conclusion that the natural nucleosides belong to the α series of glycosides. This view may be correct, but in the absence of all other evidence, one would hesitate to declare this deduction perfectly conclusive. Hence it seemed desirable to search for additional data that would give more force to the above conclusion or would compel its rejection.

With this aim in view it was attempted to obtain more information regarding the action of the nucleosidases present in the animal tissues. It has been mentioned already that glycosidases of plant origin possess a selective hydrolytic aptitude for only one form of

glucosides and Fischer and Nobel[4] have demonstrated that the glycosidases of the animal tissues were capable of hydrolyzing only one form of the glucosides, namely the β-form.

Hence the α and β forms of methylxylose and methylarabinose were prepared and added to a solution containing the active nucleosidases. Methylribose could not be obtained in crystalline form, and therefore the α and the β forms could not be separated one from another. The efficiency of the enzyme was always tested on nucleosides. To our surprise all tissue extracts failed to act on any one of the pentosides, the ribosides included. The action of the enzyme was tested by the optical method and by the reducing power of the solution for Fehling's solution.

Thus the present experiments failed to contribute to the knowledge of the structure of the nucleosides, but have furnished new information regarding the nature of nucleosidases showing that they possessed a greater degree of specificity than is known to be the property of many glycosidases.

EXPERIMENTAL PART.

Organ plasma was prepared in the manner described in a previous communication of Levene and Medigreceanu.[5] All other details of the experiments were the same as there described.

α-METHYLARABINOSIDE EXPERIMENTS.

In neutral phosphate solution (1 per cent).

Experiment with Extract of Intestinal Mucosa.

I, 16, '11. Enzyme solution, 1 cc.
α-Methylarabinoside solution, 3 cc.

Control: Enzyme solution, 1 cc.
Phosphate solution, 3 cc.

	10 min.	24 hrs.	48 hrs.	96 hrs.
Experiment:	+4.87	+4.86	+4.86	+4.86
Control:	0.00	0.00	0.00	0.00

[4] *Sitzungsberichte Berliner Akad.*, v, p. 73, 1896.
[5] This *Journal*, ix, p. 65, 1911.

Experiment with Pancreas Plasma.

I, 16, '11. Enzyme solution, 1 cc.
α-Methylarabinoside solution, 3 cc.

Control: Enzyme solution, 1 cc.
Phosphate solution, 3 cc.

	10 min.	24 hrs.	96 hrs.
Experiment:	+5.00	+4.97	+4.96
Control:	+0.06	+0.06	+0.06

α-METHYLXYLOSIDE EXPERIMENTS.

In neutral phosphate solution.

Experiment with Extract of Intestinal Mucosa.

I, 16, '11. Enzyme solution, 1 cc.
α-Methylxyloside solution, 3 cc.

Control: Enzyme and phosphate solution,
see α-methylarabinoside experiment.

10 min.	24 hrs.	96 hrs.
+2.90	+2.90	+2.89

Experiment with Pancreas Plasma.

I, 16, '11. Enzyme solution, 1 cc.
α-Methylxyloside solution, 3 cc.

Control: Enzyme and phosphate solution,
see α-methylarabinoside experiment.

10 min.	24 hrs.	96 hrs.
+3.00	+2.98	+2.98

α-METHYLGLUCOSIDE EXPERIMENTS.

In neutral phosphate solution (1 per cent).

Experiment with Extract of Intestinal Mucosa.

II, 9, '11. Enzyme solution, 1 cc.
Glucoside, 5 per cent, 3 cc.

Control: Enzyme and phosphate solution,
see maltose experiment.

10 min.	18 hrs.	120 hrs.
+3.08	+3.06	+3.05

Experiment with Pancreas Plasma.

II, 9, '11. Enzyme solution, 1 cc.
Glucoside, 5 per cent, 3 cc.

Control: Enzyme and phosphate solution, see maltose experiment.

10 min.	18 hrs.	120 hrs.
+3.15	+3.16	+3.16

Experiment with Kidney Plasma.

II, 9, '11. Enzyme solution, 0.5 cc.
Glucoside, 5 per cent, 3.0 cc.
Phosphate solution, 0.5 cc.

Control: Enzyme and phosphate solution, see maltose experiment.

10 min.	18 hrs.	120 hrs.
+3.00	+2.98	+3.02

AMYGDALIN EXPERIMENTS.

In neutral phosphate solution (1 per cent).

Experiments with Extract of Intestinal Mucosa.

Experiment I. II, 17, '11. Enzyme solution, 1 cc.
Amygdalin, 7 per cent, 3 cc.

Control (1): Enzyme solution, 1 cc.
Phosphate solution, 3 cc.

Control (2): Amygdalin solution, 7 per cent.

	10 min.	24 hrs.	96 hrs.	144 hrs.
Experiment:	−1.10	−1.24	−1.29	−1.32
Control (1):	0.00	0.00	0.00	0.00
Control (2):	−1.48	−1.70	−1.90	−2.00

Experiment II. II, 20, '11. Enzyme solution, 1 cc.
Amygdalin, 10 per cent, 3 cc.

Control (1): Enzyme and phosphate solution see preceding experiment.

Control (2): Amygdalin solution, see preceding experiment.

10 min.	24 hrs.	96 hrs.	300 hrs.
−1.47	−1.62	−1.65	−2.05

Reduced Fehling's solution.

Experiments with Pancreas Plasma.

Experiment I. II, 17, '11. Enzyme solution, 1 cc.
Amygdalin, 7 per cent, 3 cc.

Control (1): Enzyme solution, 1 cc.
Phosphate solution, 3 cc.

Control (2): Amygdalin solution, see experiment with extract of intestinal mucosa.

	10 min.	24 hrs.	48 hrs.	72 hrs.	200 hrs.
Experiment:	−1.05	−1.10	−1.25	−1.35	−1.43
Control (1):	+0.06	+0.06	+0.06	+0.06	+0.06

Experiment II. II, 20, '11. Enzyme solution, 1 cc.
Amygdalin, 10 per cent, 3 cc.

Control (1): Enzyme and phosphate solution, see preceding experiment.

Control (2): Amygdalin solution, see experiment with extract of intestinal mucosa.

	10 min.	24 hrs.	96 hrs.	300 hrs.
Experiment:	−1.45	−1.62	−1.85	−2.12

Experiment with Kidney Plasma.

Experiment I. II, 17, '11. Enzyme solution, 0.5 cc.
Amygdalin, 7 per cent, 3 cc.
Phosphate solution, 0.5 cc.

Control (1): Enzyme solution, 0.5 cc.
Phosphate solution, 3.5 cc.

Control (2): Amygdalin solution, see experiment with extract of intestinal mucosa.

	10 min.	24 hrs.	72 hrs.	200 hrs.
Experiment:	−0.90	cloudy	−1.14	−1.20
Control (1):	−0.04	cloudy	−0.03	−0.03

Experiment II. II, 20, '11. Enzyme solution, 0.05 cc.
Amygdalin, 10 per cent, 3 cc.
Phosphate solution, 0.5 cc.

Control (1): Enzyme and phosphate solution, see preceding experiment.

Control (2): Amygdalin solution see experiment with extract of intestinal mucosa.

10 min.	24 hrs.	72 hrs.	120 hrs.	500 hrs.
−1.30	cloudy	−1.60	−1.80	−2.00

Did not reduce Fehling's solution.

β-METHYLXYLOSIDE EXPERIMENTS.

In neutral phosphate solution (1 per cent).

Experiment with Extract of Intestinal Mucosa.

I, 16, '11 Enzyme solution, 1 cc.
β-Methylxyloside solution, 3 cc.

Control: Enzyme and phosphate solution, see α-methyl-arabinoside experiment.

10 min.	24 hrs.	96 hrs.
−2.60	−2.60	−2.56

Experiment with Pancreas Plasma.

I, 16, '11. Enzyme solution, 1 cc.
β-Methylxyloside solution, 3 cc.

Control: Enzyme and phosphate solution, see α-methyl-arabinoside experiment.

10 min.	24 hrs.	96 hrs.
−2.00	−2.00	−1.98

β-METHYLARABINOSIDE EXPERIMENTS.

In neutral phosphate solution (1 per cent).

Experiment with Extract of Intestinal Mucosa.

I, 16, '11. Enzyme solution, 1 cc.
β-Methylarabinoside solution, 3 cc.

Control: Enzyme and phosphate solution, see α-methyl-arabinoside experiment.

10 min.	24 hrs.	96 hrs.
+0.72	+0.68	+0.68

Experiment with Pancreas Plasma.

I, 16, '11. Enzyme solution, 1 cc.
β-Methylarabinoside solution, 3 cc.

Control: Enzyme and phosphate solution, see α-methylarabinoside experiment.

10 min.	24 hrs.	96 hrs.
+0.68	+0.64	+0.63

METHYLRIBOSIDE EXPERIMENTS.

In neutral phosphate solution (1 per cent).

Experiment with Extract of Intestinal Mucosa.

II, 2, '11 Enzyme solution, 1 cc.
Methylriboside solution, 1 cc.
Phosphate solution, 3 cc.

Control: Enzyme solution, 1 cc.
Phosphate solution, 3 cc.

	10 min.	24 hrs.	48 hrs.	96 hrs.
Experiment:	−0.39	cloudy	−0.40	−0.40
Control:	0.00	cloudy	0.00	0.00

Experiment with Kidney Plasma.

II, 2, '11. Enzyme solution, 0.5 cc.
Methylriboside solution, 1.5 cc.
Phosphate solution, 2.5 cc.

Control: Enzyme solution, 0.5 cc.
Phosphate solution, 4. 0 cc.

	10 min.	48 hrs.	144 hrs.
Experiment:	−0.50	−0.54	−0.55
Control:	−0.06	−0.06	−0.06

Experiment with Heart Muscle Plasma.

II, 2, '11, Enzyme solution, 0.5 cc.
Riboside solution, 1 cc.
Phosphate solution, 2.5 cc.

Control: Enzyme solution, 0.5 cc.
Phosphate solution, 3.5 cc.

	10 min.	24 hrs.	144 hrs.
Experiment:	−0.34	−0.37	−0.38
Control:	−0.02	−0.02	−0.02

At the end of the methylriboside experiments, none of the solutions reduced Fehling's solution. However, after the solution had been boiled with H_2SO_4 the sugar test was positive.

d-RIBOSE MUTAROTATION EXPERIMENT.

IV, 26,'11. Ribose solution, 10 per cent., in 2 dm. long observation tube. Temperature 0°C.

	15 min.	18 min.	25 min.	30 min.	40 min.	45 min.	60 min.
Observer I :	−2.73		−2.80		−2.98		
Observer II:		−2 72		−2.85		−3.01	−3.05

	18 hrs.	20 hrs.	24 hrs.	
Observer II:	−3.44	−3.44	−3.86	(Room temperature)

Reprinted from The Journal of Biological Chemistry, Vol. XI, No. 4, 1912

THE TOXICITY OF SUGAR SOLUTIONS UPON FUNDULUS AND THE APPARENT ANTAGONISM BETWEEN SALTS AND SUGAR.

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(Received for publication, March 26, 1912.)

In 1901 the writer pointed out the fundamental difference in the behavior of electrolytes and non-conductors in regard to antagonistic action on the eggs of Fundulus.[1] While it was to a large extent possible to antagonize the toxic action of one electrolyte by another it was impossible to antagonize the toxic action of an electrolyte by a non-electrolyte. Gies and I found an apparent exception to this rule in the case of the salts of heavy metals, *e.g.*, $ZnSO_4$ which could be antagonized by an excess of cane sugar,[2] and Sumner found afterwards that the toxic action of $CuSO_4$ could also be deferred through the addition of cane sugar.[3] We were inclined to explain this apparent exception on the assumption of the formation of saccharates with a diminution in the concentration of the free metal ions. In the case of the antagonization of one salt by another we are dealing with a common action of both salts upon one or several colloids on the surface of the organism.[4]

The writer recently made experiments on the toxic action of sugars on Fundulus, the results of which, at first sight, seemed to speak in favor of the possibility of an antagonization of the toxic action of sugars by salts. A more thorough analysis, however, showed that in this case the real antagonism was between two elec-

[1] *Amer. Journ. of Physiol.*, vi, p. 411, 1902.
[2] *Pflüger's Archiv*, xciii, p. 246, 1902.
[3] *Amer. Journ. of Physiol.*, xix, p. 61, 1907.
[4] *Science*, xxxiv, p. 653, 1911.

trolytes. The method employed was identical with that used in the previous experiments by Mr. Wasteneys and the writer. A series of sugar solutions was prepared, 500 cc. of each, and six Fundulus were put into each of these solutions, after having been washed repeatedly in fresh and distilled water. The number of fish which had survived the treatment was ascertained daily.

Tables I and II give the records of two simultaneous experiments, one with various concentrations of cane sugar alone, the other with the same concentrations of cane sugar solutions made up in a $\frac{M}{8}$ solution of NaCl + KCl + $CaCl_2$ (in the usual proportion) instead of in water.

A comparison of the two tables gives the typical picture of an antagonistic action; while in the pure cane sugar solutions all the fish were dead on the seventh day, in the solutions of cane sugar

TABLE I.

Number of surviving fish in various concentrations of cane sugar in water.

AFTER DAYS	CONCENTRATION OF CANE SUGAR							
	M/128	M/64	M/32	M/16	M/8	M/4	M/2	M/1
1	4	4	4	6	6	4	6	5
3	4	2	3	6	6	3	1	0
5	2	0	2	1	0	0	0	
7	0		0	0				

TABLE II.

Number of surviving fish in various concentrations of cane sugar in $\frac{M}{8}$ NaCl, KCl, $CaCl_2$

AFTER DAYS	CONCENTRATION OF CANE SUGAR							
	M/128	M/64	M/32	M/16	M/8	M/4	M/2	M/1
1	6	6	6	6	6	6	6	5
3	6	6	6	6	6	4	4	0
5	6	6	6	6	6	0	0	
7	6	6	5	6	4			
9	6	5	3	0	0			
11	6	5	3					
13	5	0	1					

made up in $\frac{M}{8}$ NaCl + KCl + $CaCl_2$ practically all the fish were alive in all the sugar solutions below $\frac{M}{4}$. Nevertheless the salt did not antagonize the toxic action of the sugar solutions in this case, but the action of a fermentation product from the sugar, namely an acid. In previous publications by Mr. Wasteneys and the writer it was shown, that the toxic effects of acid on Fundulus could be antagonized by salts, and that the concentration of acid the toxic action of which can be antagonized, is not inconsiderable.[5] Table III gives the maximum concentration of acid which is antagonized by a mixture of NaCl + KCl + $CaCl_2$ of various concentration.

TABLE III.

CONCENTRATION OF THE MIXTURE OF NaCl, KCl AND $CaCl_2$	MAXIMAL QUANTITY OF $\frac{N}{10}$ HCl WHICH THE FISH WILL RESIST IN THESE SOLUTIONS
	cc.
0	0.1–0.2
$\frac{M}{100}$	0.2
$\frac{M}{16}$	0.5
$\frac{M}{8}$	1.0
$\frac{M}{6}$	1.2–1.4
$\frac{M}{\frac{3}{8}}$	0.8
$\frac{M}{2}$	0.6
$\frac{M}{\frac{5}{8}}$	0.3
$\frac{M}{\frac{6}{8}}$	0.2

The sugar solutions with and without salt soon become turbid and later almost opaque and an examination showed that the solution was teaming with bacteria. A titration of the sugar solutions with NaOH gave a rather high degree of acidity as was to be expected. Twenty-five cubic centimeters of the $\frac{M}{\frac{3}{2}}$ cane sugar solution in $\frac{M}{8}$ NaCl + KCl + $CaCl_2$ required on the ninth day 7.5 cc. $\frac{N}{100}$ NaOH for neutralization. The toxic action of the sugar solution on the fish was therefore due in part not to the sugar but to a fermentation product, namely an acid. That the fish died of acid poisoning was also indicated by their external appearance. As I pointed out in a previous paper the epidermis

[5] *Biochem. Zeitschr.*, xxxiii, p. 489, 1911; xxxix, p. 167, 1912.

becomes white in the case of acid poisoning, and the fish which died in the sugar solutions became white before death occurred.

Similar results were obtained in experiments with dextrose, as Tables IV and V will indicate. The experiments represented in these two tables were made simultaneously.

TABLE IV.

Number of surviving fish in various concentrations of dextrose in water.

AFTER DAYS	CONCENTRATION OF DEXTROSE									
	M 128	M 64	M 32	M 16	M 8	M 4	3M 8	M 2	3M 4	M
1	6	6	6	6	6	6	6	6	0	0
3	5	4	4	4	0	0	0	0		
4	0	0	0	0						

TABLE V.

Number of surviving fish in various concentrations of dextrose in $\frac{M}{8}$ NaCl + KCl + $CaCl_2$.

AFTER DAYS	CONCENTRATION OF DEXTROSE									
	M 128	M 64	M 32	M 16	M 8	M 4	3M 8	M 2	3M 4	M
1	6	6	6	6	6	6	6	6	6	0
3	6	6	6	6	6	6	6	2	0	
4	5	6	6	6	5	6	6	0		
5	2	6	4	2	5	5	3			
7	0	0	0	0	0	0	0			

The dextrose solutions were less toxic when they were made up in $\frac{M}{8}$ Ringer solutions than when made up in H_2O. As was to be expected the dextrose solutions soon became turbid and opaque and contained a considerable amount of free acid. Again the inference was unavoidable that the antagonism in this case existed between the free acid and the salt and not between the sugar and the salt, and this surmise was supported by the fact that the epidermis of the fish showed the whiteness characteristic for the effect of acid. This idea that the acid and not the sugar solution killed the fish could be put to a further test by a comparison of the toxic action of sugar solutions which were allowed to ferment and sugar

solutions which were renewed sufficiently often to prevent a high concentration of acid through the action of bacteria. Into each of two dishes with 500 cc. $\frac{M}{8}$ dextrose six Fundulus were put. The one dish remained unaltered; the fish of the other dish were transferred into a fresh $\frac{M}{8}$ dextrose solution every twenty-four hours. In these latter solutions fermentation began also and some acid was formed, but this solution remained clear and the amount of acid was too small to do much harm. Care was taken that the new sugar solutions always had the same temperature as the old ones. In the dextrose solution which was not changed all the fish were dead after four days, as in the previous experiments, and they died with the symptoms of acid poisoning. The six fish which were transferred into a fresh $\frac{M}{8}$ dextrose solution every day are still alive and apparently normal today, on the twenty-sixth day of the experiment. This experiment was repeated with the same result.

A similar experiment was started with an $\frac{M}{8}$ solution of cane sugar. Four dishes, each with 500 cc. $\frac{M}{8}$ cane sugar, were prepared and six Fundulus put into each dish. In two dishes the solution was not renewed, the fish from the other two solutions were transferred every day into a fresh $\frac{M}{8}$ solutions of cane sugar.

In the dishes in which the cane sugar solution was not renewed the fish were all dead after three and five days respectively. The fish which were transferred every day are partly alive today after fifteen days, in one dish five out of the six put there originally are still alive and two in the other dish.

These experiments leave no doubt that at least part of the toxic effects of the sugar solutions is due to the formation of acid and that the antagonism expressed in Tables I and II and IV and V is the antagonism between acid and salts.

It became a matter of interest to find out to what extent a pure sugar solution may be called toxic for these fish. For this purpose six fish each were put into a $\frac{M}{16}$, $\frac{M}{8}$, $\frac{M}{4}$ and $\frac{M}{2}$ solution of cane sugar and the solution renewed each day. Table VI gives the results.

The rapid death in the $\frac{M}{2}$ and $\frac{M}{4}$ cane sugar solution cannot be ascribed to a product of fermentation, *e.g.*, acid; here we are possibly dealing with a direct action of the sugar. The $\frac{M}{16}$ and $\frac{M}{8}$ solutions, however, behave almost like an indifferent salt-free solution.

TABLE VI.

Number of surviving fish in various concentrations of cane sugar.

After days	Concentration of cane sugar			
	$\frac{M}{2}$	$\frac{M}{4}$	$\frac{M}{8}$	$\frac{M}{16}$
1	6	6	6	6
2	6	6	5	4
3	5	6	5	1
4	5	6	4	0
7	4	5	1	
9	4	4	0	
11	4	4		

The writer is not in a position to judge whether or not these results can be applied to the interpretation of the symptoms of patients with glycaemia. During the experiments the fish were not fed and therefore did not take up any salts.

SUMMARY OF RESULTS.

1. Fundulus live longer in solutions of cane sugar and dextrose made up in a $\frac{M}{8}$ solution of NaCl + KCl + $CaCl_2$ than in the same sugar solution without salts.

2. It is shown that in these solutions (through bacterial action) a considerable amount of acid is formed and that the apparent antagonism between sugar and salt is in reality a case of antagonism between acid and salt.

3. This conclusion is corroborated by the observation that $\frac{M}{8}$ solutions of dextrose or cane sugar, which kill the fish in a few days become almost harmless if the solution is renewed every twenty-four hours, whereby the multiplication of bacteria and the formation of acid is considerably diminished.

4. These experiments also show that $\frac{M}{8}$ or weaker solutions are in themselves nearly harmless and that their apparent toxicity upon Fundulus is due to the formation of acid or other products under the influence of bacteria. Concentrations of sugar equal to or greater than $\frac{M}{4}$ are, however, harmful independently of the acid formation by bacteria.

PHARYNGEAL INSUFFLATION, A SIMPLE METHOD OF ARTIFICIAL RESPIRATION

A PRELIMINARY NOTE *

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There are numerous occasions in the practice of surgery and internal medicine, as well as in the occurrences of accidents and intoxication in modern industrial life, in which successful artificial respiration would doubtlessly prove life-saving; the development of a simple but efficient method is therefore certainly a crying need. The well-known Sylvester and the Schäfer methods of artificial respiration are simple enough, but they are far from being reliably efficient. I shall not discuss here their merits. The usual method of artificial respiration of the physiologic laboratories is, on the other hand, efficient enough, but it requires tracheotomy and some surgical training to perform it. The method of intratracheal insufflation of Meltzer and Auer requires no surgical procedure and is, as I may emphatically state, absolutely efficient, but it requires the aid of a medical man, and of one who is already trained in the introduction of the intratracheal tube, and it requires also some sort of apparatus to perform the insufflation safely and successfully; it is highly probable that in most of the emergency cases neither will be at hand.

Serving on the Commission on Resuscitation from Electric Shock, appointed by the American Medical Association,[1] I became interested in this problem and began to study its solution experimentally. I now have reason to believe that I have already attained very promising results. Without discussing the question whether the method which I have developed should and would be recommended by the commission as the one which promises the best results, it seemed to me advisable to present it early to the medical profession. The

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1. See THE JOURNAL A. M. A., Jan. 27, 1912, p. 282.

method was studied and found efficient on four species of animals. But its real usefulness will be established only after standing the test in its application on human beings, and the final judgment will have to come from the physician and not from the experimenter in the laboratory.

The method in its simplest form consists in (1) the introduction of a catheter into the pharynx; (2) pulling out of the tongue; (3) pressing the suprahyoid region against the roof of the mouth; (4) putting the abdomen under constant pressure, and (5) connecting the catheter with bellows.

When, during a compression of the bellows, air is driven into the pharynx, it can escape through the mouth, the nose, into the stomach and into the lungs. The ready return of the insufflated air through the mouth is restricted in this method by pressing the fleshy part of the tongue against the roof of the mouth. The escape through the nose is somewhat restricted, partly by the pressure against the roof of the mouth which also raises the soft palate, and partly by the presence of the catheter in the pharynx which also assists in raising the soft palate as well as the uvula. The escape into the stomach may occur readily, if the air-pressure within the pharynx caused by the insufflation is considerable and the pressure exerted on the suprahyoid region is so strong as to prevent completely, or restrict excessively, the escape of the surplus air through the mouth. But this escape into the stomach can be entirely prevented, or restricted to a harmless minimum, by putting the abdomen under sufficient external pressure. Under these circumstances the surplus air in the pharynx during each insufflation readily enters into the lungs in a quantity and with a force sufficient to perform an efficient inspiration which, on account of the pressure on the abdomen, consists, in this case, chiefly in distention of the thorax with but little descension of the diaphragm. The heaving of the chest serves, in this case, as an indicator of the extent of inspiration. The latter, as may be pointed out here, is controlled by the degree of pressure exerted on the suprahyoid region, that is, the more pressure is here exerted, which means less escape of air through the mouth, the stronger is the inspiration. The expiration is accomplished during the intermission of the insufflation, as in normal respiration,

by the elasticity of the thoracic wall, striving to return to its normal equilibrium.

The method just described, the efficiency of which has been studied by me experimentally, contains nothing which even a layman could not grasp and perform, and it requires of apparatus only such simple contrivances as bellows, a catheter and some load for the abdomen, simple contrivances which could be kept in readiness at places where emergencies under discussion are liable to happen. In another series of experiments, however, I have studied the efficiency of this method, with the addition of another element, namely, a tube introduced into the stomach. Under these conditions no pressure on the abdomen is necessary, because all surplus air which may enter during insufflation through the esophagus alongside the tube into the stomach, escapes readily outside through the stomach-tube. The stomach-tube thus prevents the accumulation of air in the stomach and serves at the same time as a safety-valve for the surplus of air-pressure within the pharynx. The experiments have shown, further, that the rhythmic entrance of air into the stomach, instead of pressing the diaphragm headward, pushes the stomach and with it the diaphragm downward and thus assists indirectly in the inspiratory movement. Under this method the air entering the lungs is well capable of pushing the diaphragm downward; the thoracic frame becomes therefore less distended and the heaving of the chest is in this method less noticeable than in the one without the stomach-tube. It must be mentioned that the simultaneous presence of two tubes in the pharynx seems to be the means, in nearly all cases, of completely preventing the escape of the insufflated air through the nose.

The methods have been developed, and sufficiently tested by experimentation on four species of animals: dogs, cats, rabbits and two monkeys. The most extensive series of experiments were carried out on dogs and cats. Since the chief object of this study was to find a method of providing efficient artificial respiration, it was best to study the efficiency of the methods on animals in which the normal respiration was completely abolished, while other functions were but little impaired. The experiments were therefore carried out chiefly on curarized animals, all of which received morphin and some receiving also ether. Without entering on details,

I may state here, briefly, that in all animals, without exception, either of the two methods was all sufficient to keep up the lives of completely curarized animals for many hours in a perfectly normal manner. In a good many experiments the thorax was widely opened transversely and the heart freely exposed. No shock was observed in any case. In several instances the artificial respiration was discontinued as long as from six to seven minutes, until the pulse disappeared completely, the ventricles showing only a faint, inefficient beat. Nevertheless, on restoring the connections for artificial respiration, heart and circulation were restored to normal within from one and a half to two minutes. Several animals were kept under the influence of curare for several hours, but were otherwise not operated on; they were permitted to recover from the influence of curare and remained perfectly well. These methods proved to be sufficient to keep up the respiration in cases also in which the medulla oblongata was completely destroyed or after complete abolition of respiration by intramuscular injection of large doses of magnesium sulphate.

It was further established that by means of pharyngeal insufflation animals were brought easily and profoundly under the influence of ether anesthesia. When the profound anesthesia continued for some time, the spontaneous respiration disappeared completely. When now the ether was discontinued, the animal remained, sometimes for half an hour and longer, without any spontaneous respiration; the heart nevertheless continued to beat regularly and improve rapidly in efficiency. Here again the pharyngeal insufflation proved to be an efficient method of artificial respiration and life-saving.

I have made, also, a few experiments on animals which were killed purposely either by etherization or by illuminating gas. In these cases the pharyngeal insufflation was not instituted until all traces of respiration and heart-beats disappeared. So far, only two recoveries can be recorded. No serious attempt, however, has yet been made to study those problems in a proper manner. In the question of resuscitation from these poisonings, other problems are involved, with which we are not concerned for the present.

My experimental studies convinced me that by means of these methods of pharyngeal insufflation, artificial

respiration can be kept up efficiently, and animals will be surely resuscitated from a purely respiratory death. Seeing no reason whatsoever why these methods should fail to accomplish similar results in human beings, I submit to physicians and surgeons my request to test the pharyngeal methods, as described here, on human beings wherever there is any need of artificial respiration; the opportunities will not be lacking. There are cases in surgery on the brain, the thorax, or on other parts of the body, in which the respiration gives out, while the heart is still beating. There are cases of respiratory insufficiency also in internal medicine, for instance in postdiphtheritic paralysis, in poliomyelitis, or in some cases of eclampsia or coma. And there are the poisonings by morphin, illuminating gas, etc., or the shocks in accidents. The pharyngeal insufflation, when carried out with care, can certainly do no harm; its execution is very simple, and it ought to be tested in any of these cases. Furthermore, I can see no objection to its being tested immediately after death from any cause whatsoever. There is always the possibility that the actual cause of death might be, in one case or another, especially in acute cases, only of a temporary nature, so that efficient artificial respiration might assist in temporizing and thus prove occasionally life-saving indeed. Such possibilities, though they may be realized only once in a thousand times, justify the making of such an attempt in each and every instance of death. It is thus that the efficiency of pharyngeal insufflation, as a means of artificial respiration in human beings, could be tested early and reliably. It is hardly necessary to point out that it would not be a fair test of the method to try it after rigor mortis had set in.

To facilitate the application of the method, I shall try to describe concisely, here, the manner of its application as I believe it ought to be used in human beings. The tube to be inserted into the pharynx should be a 15 American (or 22 French) catheter. If there is no catheter at hand, any tube of a similar diameter will serve the purpose, but it ought to possess sufficient resistance and should have at least one fair-sized lateral opening near its end. The catheter should be introduced into the pharynx about 5½ inches from the teeth. The tongue should be pulled out and held by a tongue-forceps, the continuation of which is, however, not

indispensable, since the bandage will later keep the tongue in proper position. Then a pad, about 1 inch long and 1 inch thick, made of gauze, cotton or any other material, should be placed under the chin on the suprahyoid region and pressed upward by means of a bandage or a folded handkerchief tied at first only moderately firmly over the head. By the suprahyoid region is meant the part of the submental region which is nearest to the hyoid region; pressure on this part presses the middle part of the tongue against the posterior part of the hard palate. The bandage over the head should be tied about over the posterior part of the parietal bones.

Now some weight should be placed on the abdomen. In unconscious individuals the abdominal wall offers no resistance and a load of from 12 to 13 pounds will be sufficient to exert the necessary pressure. A weight of from 6 to 7 pounds was sufficient for fairly large dogs. The pressure on the abdomen offers the further advantage that it drives the blood from the abdominal viscera to the heart and to the brain and the medulla where it can do greater service.

The bellows, which need not be of very large size, are now connected with the catheter and the manipulation may begin. Care should be taken that at the beginning the compression should not be made too forcibly and rapidly and not oftener than ten to twelve times per minute. If each compression causes only a slight heaving of the chest the bandage over the head should be tightened and if the heaving appears to be too strong the bandage should be loosened correspondingly. Here, as in the method of intratracheal insufflation, too much pressure and careless handling may do harm. The compression should never be made so tight as to make the escape of air through the mouth impossible or very difficult.

The handling of the bellows is often fatiguing; but the effort can be greatly reduced by fastening one handle by means of a screw-clamp to a table and making the other handle several feet long; the working of the bellows is then very easy.

The introduction of a tube into the stomach permits the handling of the insufflation with greater force, more efficiency, and even greater safety. In some cases the

use of the stomach-tube might be even indispensable, when, for one reason or another, no weight can be placed on the abdomen. The stomach-tube should not be too small in diameter, should have two lateral openings near its end and should be introduced at least sixteen inches from the teeth. It should be introduced before the pharyngeal tube. There is no objection to having both devices together—the stomach-tube and the weight on the stomach.

I shall add that in my experiments the insufflation was just as efficient when the animal was lying on the stomach with the head hanging downward. Under these circumstances it is, however, necessary to make a provision for keeping the tubes in place; otherwise they might slip out.

In the foregoing the bellows were relied on as a source of pressure for the insufflation. It is evident, however, that any other source of interrupted or continuous pressure could be employed. It is especially worth while to mention here that in the absence of bellows an oxygen tank could be used for this purpose. It is necessary only to insert a T tube in the connection between the oxygen tank and the catheter. An attendant should then close and open the vertical, open branch of that tube about twelve times per minute; the closing will cause an inspiration and the opening an expiration. The closure should last about two and the opening about three seconds. In order to prevent too much pressure it is advisable to introduce another T tube which should be connected with a safety valve. The latter is prepared in the easiest and simplest manner by having mercury in a double-necked bottle or a bottle closed with a stopper having two perforations. Through one of these openings a graded tube is introduced and submerged about 15 to 20 mm. under the surface of the mercury. That means that the oxygen will never enter the pharynx with a pressure higher than 15 or 20 mm. mercury.

Finally, it is evident that the pharyngeal insufflation ought to be capable of rendering good service in the asphyxia of the new-born. After introducing proper-sized catheters into the stomach and the pharynx and after applying properly a bandage around chin and head, the pharyngeal catheter should be connected simply

with a rubber bulb of an atomizer and the artificial respiration can be started. This simple arrangement will probaby be sufficient also for the resuscitation of babies 1 or 2 years old.

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Reprinted from The Journal of the American Medical Association
May 11, 1912, Vol. LVIII, pp. 1413-1415

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Reprinted from The Journal of Experimental Zoölogy, Vol. 12, No. 4
May, 1912

ON THE ADAPTATION OF FISH (FUNDULUS) TO HIGHER TEMPERATURES

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I. INTRODUCTION

It is a well known fact that organisms can stand a higher temperature if the latter is raised gradually than if it is raised suddenly. This phenomenon is referred to in biology as a case of adaptation. Dallinger states that he succeeded in adapting certain protozoa to a temperature of 70° by gradually raising their temperature during several years.

Schottelius had found that colonies of Micrococcus prodigiosus when transferred from a temperature of 22° to that of 38° no longer formed pigment and trimethylamin. When transferred back to the temperature of 18° to 22° the formation of pigment and of trimethylamin was resumed. After the cocci had been cultivated for ten or fifteen generations at 38° they failed to form pigment even when transferred back to 22°.

These experiments became the starting point for similar experiments by Dieudonné. He used Bacillus fluorescens putidus which forms a fluorescein pigment and trimethylamin. The optimal temperature for this bacillus is 22°. At 35° it grew but did not form pigment or trimethylamin. At 37°.5 growth ceased. Retransferred to 22° pigment and trimethylamin were again formed.

Dieudonné[1] exposed a culture of this bacillus to 35°. After twenty-four hours a second culture was taken from this and also kept at 35°, and this process was repeated each day. The fifteenth generation thus cultivated at 35° began to form some pigment and from the eighteenth generation on, at 35° the formation

[1] Dieudonné, Arb. aus dem kaiserl. Gesundheitsamte, vol. 9, p. 492, 1894.

of pigment and trimethylamin had become as good as that in the cultures kept at 22°. The organisms had, therefore, become 'adapted' to a temperature of 35° which at first was unfavorable.

Dieudonné did not succeed in causing pigment formation in this bacillus at a higher temperature than 35° although the bacillus finally grew at a temperature of 41°.5. He obtained similar results in experiments on other pigment-forming bacteria. Davenport and Castle[2] made experiments with tadpoles of frogs. One lot of eggs and tadpoles was kept at about 15°, a second lot for twenty-eight days at 25°C. While the tadpoles raised and kept at 15° went into heat rigor at 40°.3C., those kept for twenty-eight days at 25° were not affected by this temperature but went into heat rigor at 43°.5. Their resistance to high temperature had therefore risen 3°.2. When the latter tadpoles were put back for seventeen days to a temperature of 15° they had lost their resistance to high temperature to some extent but not completely, since they went into heat rigor at 41°.6. The authors suggest that this adaptation to a higher temperature is due to a loss of water on the part of the protoplasm. They assume that the rise in temperature causes a comparative acceleration in the excretion of water on the part of the tadpoles. The hypothesis of these authors is based upon the fact, that spores of bacteria are more resistant to high temperatures than the bacteria. While this is a fact, nothing in the experiments of Davenport and Castle proves that the amount of water in the tadpoles is diminished by the rise in temperature. The idea of Davenport and Castle was put to a direct test by Kryz.[3] He kept frogs, toads and salamanders at room temperature, and at temperatures as high as 40°C., for a number of days or weeks and tested the coagulation temperature of the muscle plasma for both ranges of temperatures. He found that the coagulation temperature was identical for the animals kept at low and those kept at high temperatures.

The following observations by Kammerer[4] indicate also an after-effect of the raising of temperature. Lacerta muralis from the

[2] Davenport and Castle, Arch. f. Entwickelungsmechanik, vol. 2, p. 227, 1896.
[3] Kryz, Arch. f. Entwickelungsmechanik, vol. 23, p. 560, 1907.
[4] Kammerer, Arch. f. Entwickelungsmechanik, vol. 30, p. 379, 1910.

cooler climate of Nieder Oesterreich darkens already at a temperature of 25° and becomes perfectly black at a slightly higher temperature. Lacerta muralis from the warmer climate of Italy remains perfectly normal at 25° and darkens only at 37°. Kammerer points out that the higher temperature at which the latter lizards had lived prevented the effects which the same rise in temperature produced in lizards that had previously lived at a lower temperature.

II. THE INFLUENCE OF THE CONCENTRATION OF THE SOLUTION UPON THE RESISTANCE OF FUNDULUS TO HIGH TEMPERATURE

We used as material for our experiment half-grown Fundulus which were caught in Long Island Sound in January and kept in a room in the laboratory, the temperature of which varied a little around 10°C. Two fish were put into each of the following solutions: H_2O, m/128, m/64, m/32, m/16, m/8, m/4, NaCl + KCl + $CaCl_2$ in the usual proportion. It was found that the higher the concentration of the solution—up to a certain limit—the longer the fish could survive in a given high temperature. The method consisted in putting a number of battery jars with 500 cc. of the solution into a large water bath the temperature of which was constantly watched and kept constant. When all the solutions had reached the desired temperature the fish were introduced. In a number of cases the experiment was continued for several days in a bacteriological thermostat. Table 1 gives the duration of life for these fish in each of the solutions for various tempera-

TABLE 1

TEMPERATURE	DURATION OF LIFE OF FUNDULUS IN								
	H_2O	m/128	m/64	m/32	m/16	m/8	m/4	$\frac{3}{8}$ m	NaCl + KCl + $CaCl_2$
25°	4 hrs.	indef.	indef.	indef.	indef.	indef.	indef.	indef.	
27°	1 hr.	2 hrs.	2 hrs.	indef.	indef.	indef.	indef.	indef.	
29°	7′	31′	50′	62′	90′	indef.	indef.	indef.	
31°	13′		25′	17′	50′	indef.	indef.	indef.	
33°	2′			6′	9′	7′	9′	80′	

tures. The term 'indefinite' means that the fish were alive and apparently normal at the end of the experiment.

These experiments were repeated with the same material with approximately identical results; 33° was as a rule the upper limit the fish could resist during the month of January. Occasionally a fish would survive a sudden exposure to a temperature of 33° in a m/4 solution of NaCl + KCl + $CaCl_2$ but such fish were no longer normal and would swim on their side.

It was next ascertained in which concentration the fish could resist the highest temperature. It was found that this concentration was m/4. In m/2 and $\frac{5}{8}$ m they were not able to resist as high a temperature as in m/4 or $\frac{3}{8}$ m solution of NaCl + KCl + $CaCl_2$. When sea-water was substituted for Ringer's solution (NaCl + KCl + $CaCl_2$) the results were the same as represented in table 1.

TABLE 2

TEMPERATURE	DURATION OF LIFE OF FUNDULUS IN								DEXTROSE
	H_2O	m/32	m/16	m/8	m/4	$\frac{3}{8}$ m	m/2	$\frac{5}{8}$ m	
29°	40′	40′	30′	40′	100′	40′	70′	40′	
31°	16′	20′	16′	20′	20′	24′	25′	18′	

The fact that the temperature which the fish could resist was the higher, the higher the concentration of the solution, suggested the possibility that the loss of water on the part of the fish increased their resistance. This explanation, however, does not agree with the observation made by Sumner and corroborated by us that Fundulus undergoes practically no change in weight when put into distilled water or when put back into sea-water. The idea that loss of water made Fundulus more resistant to heat could be tested by the substitution of sugar solution for Ringer's solution or sea-water. It was found that dextrose solutions were not able to protect the fish, in fact such solutions were little if any better than H_2O (table 2).

The duration of life of the fish in the dextrose solutions is about identical with that in distilled water. This excludes the suggestion that osmotic phenomena determine the influence of the con-

centration found in the experiments with Ringer solution or with sea-water; especially if we consider the fact that Loeb found that Fundulus can live for over a month in an m/8 solution of dextrose at ordinary room temperature (if the solution is renewed every day).

The difference between the results expressed in tables 1 and 2 suggested that the protective action of the Ringer solution was of the nature of a specific salt action. We, therefore, tested the idea whether or not other salt solutions, *e.g.*, NaCl or $CaCl_2$ could afford the same protection. It was found that this was true to a slight degree in the case of NaCl but not in the case of $CaCl_2$. Table 3 may serve as an example.

TABLE 3

TEMPERATURE	DURATION OF LIFE OF FUNDULUS IN								
	H_2O	m/64	m/32	m/16	m/8	m/4	½ m	m/2	NaCl
29°	30′	30′	40′	50′	indef.	120′	120′	30′	
	H_2O	m/64	m/32.	m/16	m/8	m/4	½ m		$CaCl_2$
29°	20′	100′	60′	90′	60′	30′	10′		

In an m/8 NaCI solution some but not all of the fish could live indefinitely at 29°; in an m/8 or m/4 solution of sea-water or Ringer solution they could all live indefinitely at 29°. We must therefore conclude that the protection which sea-water or a Ringer solution gives Fundulus against a high temperature is due to a specific effect of the combination of the three salts NaCl, KCl and CaCl in the right proportion. The idea presented itself that this protective action of the salts was the expression of an antagonism between the salts and a substance produced at a great velocity at a higher temperature, *e.g.*, an acid. Experiments, to be discussed a little further on, on the immunization of fish against a high temperature eliminate this possibility.

Experiments were tried on tadpoles and on a species of fresh water fish to ascertain whether these animals could resist high

temperatures better in an m/8 Ringer solution than in tap water or weaker Ringer solutions. No positive results were obtained.

III. THE ADAPTATION OF FUNDULUS TO HIGH TEMPERATURES

Fish from the cold room (10° to 14°C.) were kept for lengths of time varying from one hour to several hours at 27°C., in an m/4 Ringer solution, and then put into H_2O, m/64, m/32, m/16, and m/8 Ringer solution at 31°. It was found that the longer they stayed at a temperature of 27° the more resistant they became to the temperature of 31°, so that finally they survived at that temperature even in distilled water.

One sees that fish that had been kept in m/4 Ringer solution for seventy-two hours lived indefinitely in 31° even in distilled

TABLE 4

PREVIOUSLY EXPOSED TO 27° IN m/4 RINGER SOLUTION FOR	DURATION OF LIFE OF FUNDULUS AT 31° IN RINGER SOLUTION				
	H_2O	m/64	m/32	m/16	m/8
0 hour	13′	40′	43′	120′ or indef.	indef.
1 hour	35′	95′	150′	102	indef.
4 hours	62′	indef.	indef.	indef.	indef.
23 hours	180′	indef.	indef.	indef.	indef.
72 hours	indef.	indef.	indef.	indef.	indef.

water. It should be stated that each experiment was accompanied by a control experiment with animals that had not been immunized and in all cases the fish die in less than an hour in 31° in solutions below m/8.

Fish kept in the cold room (10° to 14°C.) and put from there directly into a solution of 35° nearly all died in a few minutes even in the optimal solution of m/4 sea-water or Ringer. Experiments were undertaken to ascertain the minimum time the fish had to be kept at 27° in m/4 sea-water in order to be rendered immune to a sudden transfer into m/4 Ringer solution at 35°.

It should be stated, however, that the fish that had been kept at 27° for sixteen and twenty-one hours did not all survive in 35°

TABLE 5

PREVIOUSLY IN THERMOSTAT AT 27°	DURATION OF LIFE OF FUNDULUS AT 35°
0 hour	3′
1 hour	5′
3 hours	3′
6 hours	3′
8 hours	6′
16 hours	indefinitely
21 hours	indefinitely
44 hours	indefinitely

while all the fish that had been kept at 27° for forty-four hours survived.

This experiment was repeated with a second set of fish with the results shown in table 6.

TABLE 6

PREVIOUSLY IN THERMOSTAT AT 27° FOR	DURATION OF LIFE OF FUNDULUS AT 35° m/4 RINGER
1 hour	2′
3 hours	2′
6 hours, 8 hours, 16 hours	partly indefinite
21 hour, 24 hours	partly indefinite, but better than previous group
32 hours, 40 hours, 48 hours, 65 hours, 72 hours	indefinite

It seems that if Fundulus are kept more than twenty-four hours in a temperature of 27° they can with impunity be put into an m/4 Ringer solution of 35°. If fish are kept from six to sixteen hours at 27° and then suddenly transferred to 35° (in m/4 Ringer solution) some of them die and some survive; and the tendency to survive increases the longer the fish are previously kept at 27°.

A series of experiments was carried out in which fish were kept in a thermostat at 30° C. for various lengths of time to test whether this accelerated their adaptation to a temperature of 35°. This was true only to a slight extent. In all these experiments the fish were suddenly transferred to an m/4 Ringer solution at 35°.

We were curious to know if these animals could also survive if suddenly transferred to a temperature of 35° in distilled water. This is indeed the case as table 7 shows.

Fundulus can become adapted to a temperature of 35° in distilled water if they are kept for two days or longer at a temperature of 27°. It seemed to make no difference whether the fish had been kept at 27° in m/4 sea-water or in distilled water.

TABLE 7

PREVIOUSLY EXPOSED TO 27° FOR	DURATION OF LIFE OF FUNDULUS IF SUDDENLY PUT INTO DISTILLED WATER OF 35° C.
0 days (control)	4′
2 days	indefinite
3 days	indefinite

Finally experiments were made to see to how high a temperature these fish could be adapted in a week. By keeping the fish at a temperature of 27° over night and raising them during the day to a gradually higher temperature we found that they could be kept at the end of the week at a temperature of 40°C., for two hours without apparent injury. At a temperature of 41° they soon suffered in their power to maintain their equilibrium. They were immune to a temperature of 40° not only in an m/4 Ringer solution, but also in an m/64 solution. The lot which was in distilled water died early during the experiment through an accident. It is probable that Fundulus once adapted to a certain temperature can stand this temperature in any concentration of a Ringer solution below m/4.

IV. THE SUMMATION OF THE IMMUNIZING EFFECTS OF SHORT PERIODS OF EXPOSURE TO HIGH TEMPERATURE

In the immunization experiments described thus far the fish had been exposed continuously for a rather long period of time to a temperature of 27°. We wanted to know if it was possible to immunize them for a higher temperature by exposing them only a short period of time each day and keeping them in the interval at a temperature of from 10° to 14°C. This would mean that the immunizing effect produced in the animal during a short exposure to a high temperature would be preserved at least twenty-four hours until the next exposure to a high temperature took

TABLE 8

DATE	DURATION AND TEMPERATURE OF EXPOSURE	
March		
7	1 hour from 17° to 31°	2 hours at 31°
8	1 hour from 17° to 33°	2 hours at 33°
9	1 hour from 18° to 35°	2 hours at 35°
11	3 hours from 11° to 37°	
12	2 hours from 17° to 37°	
13	2 hours from 16° to 37°	
14	2 hours from 17° to 37°	
15	2 hours from 17° to 37°	2 hours at 37°
16	2 hours from 17° to 37°	2 hours at 37°
18	2 hours from 17° to 38°	1½ hours at 38°
19	2 hours from 18° to 38°	2 hours at 38°
20	2 hours from 19° to 39°	2 hours at 39°

place; and would be added to the immunizing effect of the next exposure to a high temperature. Table 8 gives the periods of exposure.

Most of the fish died on the third day when the temperature was raised only to 35°. For this reason we did not dare to expose the fish for more than a few minutes to a temperature of 37° on the 11th, 12th, 13th and 14th of March. The fish were exposed to a higher temperature for not more than four hours on one day. We have seen that an exposure of four hours in itself does not suffice to create immunity to a temperature of 35° or above. Hence the fact that these fish were finally able to resist a

temperature of 39° indicates a cumulative effect of the different exposures to a higher temperature. In other words, each heating increased the immunity and this gain was not lost during one or two days.

V. THE IMMUNITY TO A HIGH TEMPERATURE IF ONCE ACQUIRED IS KEPT FOR MORE THAN FOUR WEEKS

In order to prove this, fish were put for various lengths of time into a thermostat at 27°, tested in regard to their immunity against high temperature and then put back into the cold room and tested again. A few examples will illustrate this. Five fish were immunized to a temperature of 39° by exposing them daily for a number of hours to an increasing temperature, until they could live in a temperature of 39°C. (in m/4 Ringer solution). The process of acclimatization extended over a period of twelve days (see previous experiment). After this they were kept for eight days constantly at a temperature of from 10° to 14°C. On the eleventh day they were put suddenly into a temperature of 31° and the temperature of the water in which they were, was brought, inside of two hours, to a temperature of 39°, and then kept at this height. A control experiment was carried on simultaneously with fish taken from the same cold room, which had not been acclimated. The solutions used were m/4 Ringer. The control fish that had not been acclimated to high temperature were dead in one and a half hour when the temperature had reached 36°. The acclimated fish kept alive for over an hour at 39° when the experiment was discontinued. In eight days, therefore, the immunity of the fish to high temperature had not diminished.

Four lots of fish had been immunized to a temperature of 35° by keeping them twenty-four ,thirty-two, forty and seventy-two hours respectively at a temperature of 27°. After this the fish were put into the cold room and kept there at a temperature ranging from 10° to 15°C., for twenty-eight days. They were then put into an m/4 Ringer solution at a temperature of 35°. Simultaneously six fish of the same lot, which had not been immunized but kept in the cold room permanently, were put into the same temperature and the same solution. Four of the latter fish died

within two minutes, the rest were dead forty minutes later. The fish that had been immunized before were, with the exception of two individuals, all alive and normal after five hours. Yet, the fact that two of the fish succumbed may be an indication that their resistance to 35° was less than immediately after immunization. It is quite possible, however, that these two fish which had been kept in small dishes for such a long time had suffered through this captivity.

This idea is supported by the fact that in a third experiment fish had kept their immunity to high temperature for thirty-three days after immunization against 35°. The immunization consisted in exposing the fish for three and six days respectively to 27°. After that they were kept in the cold room for thirty-three days. When after that time subjected to a temperature of 35° they remained perfectly normal for five and a half hours, when the experiment was discontinued.

We made a large number of experiments in which the duration of the immunity against high temperatures was tested sooner after the process of immunization than in the above mentioned experiments. In all these experiments it was found that the fish did not lose their immunity against temperatures of 39° and 35° respectively if they were put into the cold room for a period of thirty-three days or less after immunization.

VI. EXPERIMENTS WITH FISH KEPT AT A CONSTANT TEMPERATURE OF 0°.4 C.

The fish which we used for this experiment were caught in January and kept since that time in a cold room in which the temperature varied between 10° and 14°C. Our experiments showed that these fish died in a rather short time when suddenly put into a diluted Ringer solution or diluted sea-water of 31°, provided that the concentration of the solution was below m/8. In an m/8 or m/4 Ringer solution or sea-water they were able to resist the temperature of 31° without any previous immunization. We put a large number of these fish in an ice chest in which the temperature remained constantly at 0.°4, and investigated at various intervals whether the resistance of these animals to a

temperature of 31° differed from that of the fish kept at from 10° to 14° (cold room). The fish put in the ice chest had previously been at a temperature of 10° for several weeks.

If we consider the behavior of the fish in an m/8 solution at 31° we notice a steady diminution of resistance among those kept at 0°.4; while among those kept at 10° to 14° the resistance increased somewhat. The latter result is not accidental. We must remember that the fish were taken in January when the temperature of the water was not far from 0°C. The long exposure to a temperature of from 10° to 14° had therefore a slight immunizing effect.

Our next task was to ascertain whether fish immunized to resist a sudden transfer to a temperature of 35° kept this immunity if put on ice just as well as if kept at a temperature of 10° for the same period. Our experiments thus far cover only an exposure of fourteen days on ice (T. = 0°.4). During this time the immunity was not diminished, as the following example will show. Fish were immunized to a sudden transfer to 35°C. by keeping them for two days at 27°. They were then put into a thermostat with a constant temperature of 0°.4 for fourteen days and put directly

TABLE 9

KEPT AT 0.4° C.	DURATION OF LIFE AT 31° IN				RINGER SOLUTION
	H_2O	m/32	m/16	m/8	
7 days	26′		104′	indef.	
19 days	13′	42′	17′	some indef.	
33 days	24′	30′	69′	80′	
41 days	22′	34′	22′	8′	

KEPT AT 10° C.	DURATION OF LIFE AT 31° IN				RINGER SOLUTION
	H_2O	m/32	m/16	m/8	
Over 7 days	20′		135′	indef.	
Over 19 days	13′	26′	52′	indef.	
Over 33 days	42′	86′	some indef.	indef.	
Over 41 days	41′	some indef.	some indef.	indef.	

into a m 4 Ringer solution of a temperature of 35° (A). Simultaneously fish which had been immunized for 35° by keeping them three days at 27° and which had then been kept at between 10° and 14° for nineteen days were also put into a m/4 Ringer solution of 35° (B). In addition two controls were made: Fish kept on ice at 0°.4 for twenty days but not previously immunized (C), and fish not previously immunized kept for several weeks at a temperature of from 10° to 14° (D) were also suddenly transferred to a temperature of 35°. Table 10 gives the result.

TABLE 10

DURATION OF LIFE OF FISH AT 35°C. IN m/4 RINGER SOLUTION

A. Immunized but kept on ice for fourteen days	B. Immunized but kept at 10° for nineteen days	C. Not immunized kept on ice for twenty days	D. Not immunized kept in cold room
Alive after 3 hours	One alive after 3 hours	Die in 2′	Die in 2′

The experiment was repeated with the same result, only those in lot A and B remained all alive. It is therefore obvious that the resistance acquired for a higher temperature (35°) is not lost or diminished if the fish are kept for two weeks on ice.

THEORETICAL

The phenomenon of adaptation considered in this paper is the fact that fish can resist a high temperature better if the latter is raised gradually than when it is raised suddenly. Physics offers us an analogy to this phenomenon in the experience that glass vessels which burst easily when their temperature is raised suddenly, remain intact when the temperature is raised gradually. This phenomenon finds its explanation in the fact that glass is a poor conductor of heat and that when the temperature is raised suddenly, *e.g.*, inside a glass cylinder, the inner layer of the cylinder expands while the outer layer, on account of the slowness of the conduction of heat, does not expand equally and the cylinder bursts.

The following idea for the explanation of the mechanism of adaptation suggests itself. The rise in temperature brings about certain changes especially in the surface of the cells or the body of the animal, whereby the latter loses its protective impermeability. If the rise in temperature occurs gradually the blood (and especially the salts of the blood or of the surrounding solution or of both) has time to repair the damage. If the rise, however, occurs suddenly then the damage done cannot be repaired quickly enough by the blood, or the salts of the surrounding solution, to prevent the death of the cell or the animal. The peculiar influence of the concentration and nature of the surrounding solution described in this paper would harmonize with this suggestion.

A second possible suggestion is that under the influence of the higher temperature a substance is formed in the animal which protects it against the effects of high temperature. The formation of this substance is also a function of time and for this reason an animal can keep alive if the temperature is increased gradually but cannot keep alive if it is increased rapidly.

Both suggestions would explain the fact that if an animal is once immunized against a high temperature it will keep this immunization, for some time at least, even if kept at a low temperature or on ice. Further experiments with which we are occupied may decide between these and other possible suggestions.

SUMMARY

1. Experiments were made with Fundulus which were caught in the winter and kept at a low temperature (from 10° to 14°C.), to find out the maximum temperature into which they could, with impunity, be transferred suddenly. It was found that the maximum temperature varied with the concentration of the sea-water or a Ringer solution; being about 25°C. for a concentration of m/128 or m/64; 27°C. for a concentration of m/32; 31°C. for a concentration of m/8, and almost 33°C. for a concentration of m/4. The latter concentration was the optimum, the resistance to high temperature decreasing again with a further rise in concentration.

2. It was found that dextrose solutions were not able to afford any protection against the effects of a sudden rise in temperature. From these and similar experiments with $CaCl_2$ solutions it follows that the protective action of sea-water or a Ringer solution against high temperature is not an osmotic but a specific effect of the salts of the sea-water.

3. It was ascertained how long it takes to immunize the fish against the harmful effects of a sudden transfer to a temperature of 35°C. It was found that by keeping the fish for thirty hours or more at a temperature of 27° they were immunized against a temperature of 35°. Often a noticeable immunizing effect was produced already by an exposure of sixteen hours or even a little less to a temperature of 27°. Fish kept for two days at 27° were able to survive if suddenly transferred to distilled water of 35°C.

4. The immunity against a temperature of 35° acquired by keeping the fish for two days at 27° is not lost or weakened if the fish are afterwards kept as long as thirty-three days at a temperature of from 10° to 14°. Our experiments have not been extended beyond this period of time.

5. The immunity against a temperature of 35°C. is also maintained if the fish are kept after the two days' exposure to 27° for two weeks at a temperature of 0°.4 C.

6. Fish immunized against a temperature of 39° and then kept at a temperature of from 10° to 14° for eleven days did not lose their immunity.

7. A longer exposure of fish to a temperature of 0°.4 may finally lower their resistance to high temperature.

8. In order to immunize fish to a temperature of 39° it is not necessary to expose them continuously to a higher temperature. An intermittent exposure to a higher temperature during a number of hours each day will bring about the same effect.

9. Various suggestions for a possible theory of these phenomena are made.

A FURTHER REPORT ON THE PRODUCTION OF EXPERIMENTAL CHRONIC NEPHRITIS IN ANIMALS BY THE ADMINISTRATION OF URANIUM NITRATE*

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In a previous report[1] I was able to show that by the administration of uranium nitrate to guinea-pigs it is possible to produce in them a condition which is analogous to various stages of chronic diffuse nephritis in man. These changes were quite constantly produced, and were found after the administration of uranium nitrate in any one of the following three ways: first, after a series of frequently repeated injections of very small doses of the drug; second, after a series of injections of moderately large doses at intervals of a considerable length of time; and third, after the injection of a single sublethal dose.

The earlier experiments were performed exclusively on guinea-pigs, but during the past three years a large number of experiments have been performed on guinea-pigs, rabbits and dogs in the hope of confirming my previous observations and of producing the condition among animals of different kinds which would be better suited for secondary experiments. In all of these types of animals it has been possible to produce lesions which are characteristic and which are very similar in practically every case. It is true that the histological picture differs considerably from that which is found in human nephritis, but the gross appearance of the kidney in advanced cases is that of granular atrophy, and the histological picture is that of an extreme and progressive fibrosis with accompanying changes in the tubules and glomeruli, so that one cannot do otherwise than describe the condition as a true chronic diffuse nephritis. It is of interest that these results are seemingly at variance with those obtained by Christian and Smith,[2] who were unable to reproduce conditions similar to those which I had previously reported in guinea-pigs, and who also found that in rabbits they obtained less satisfactory results with uranium nitrate than with potassium bichromate.

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*Manuscript submitted for publication March 15, 1912.

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*A portion of these experiments were performed under a grant from The Rockefeller Institute for Medical Research.

1. Dickson, E. C.: A Report on the Experimental Production of Chronic Nephritis in Animals by the Use of Uranium Nitrate, THE ARCHIVES INT. MED., 1909, iii, 5 and 6, 375.

2. Smith, R. M.: Experimental Chronic Nephritis. THE ARCHIVES INT. MED., 1911, viii, 481.

Before detailing the protocols of the cases which I wish to report, it would seem advisable to describe briefly the characteristic histological picture of the lesions, since they occur so constantly, not only in the kidneys of the different animals of the same kind and of the same experimental series, but also in the kidneys of animals subjected to different experimental procedures, and, indeed, even in the kidneys of the different kinds of animals.

The kidneys all show proliferation of the interstitial connective tissue, which is constantly much more marked and much more fibrous in the inner zone of the cortex. From this inner fibrotic area rays of more cellular connective tissue radiate out toward the surface, and in the more severe cases which have granular surfaces these rays extend to the surface and correspond to the small depressions on it. In the majority of cases the processes are fairly well restricted to the medullary rays, but in the more severe cases, especially in the kidneys of dogs, there is a marked, diffuse proliferation of connective tissue all through the cortex, so that practically no normal appearing tissue remains. In all cases the connective tissue is quite cellular in the outer zone of the cortex, and in many cases there are large areas of dense round cell infiltration in the region of the larger vessels and around some of the glomeruli. In many cases there is a more or less well marked proliferation of connective tissue in the outer portion of the medulla.

The glomerular changes vary considerably, depending on the situation of the glomeruli in the cortex and the severity of the fibrosis of the kidney; and they are always much more severe in the inner zone of the cortex where the connective tissue proliferation is most marked. In every case the glomerular lesions would seem to be of secondary nature, since in the acute uranium nephritis the glomeruli show practically no lesions.[3] The most constant change is that of pyknosis and fragmentation of the nuclei in the tuft. In some more severe cases there seems to be an increase in the connective tissue elements in the tufts, and in a few cases, especially in rabbits, there is distinct thickening of the capillary walls in the tufts, and a deposit of some hyaline substance in some of the tufts. In the areas where there is marked fibrosis, many of the glomeruli are completely collapsed. In practically all cases there is more or less marked thickening of the glomerular capsules, and in many there is proliferation of the capsular epithelium. In many cases there is exudation into the capsular spaces, and in some there is also detached epithelium which seems to be crowded into them from the adjoining tubule. In many cases the capsules are distended and form quite large cysts. In those more

3. Christian has described the occurrence of small, oval or round hyaline bodies in the glomerular tufts of rabbits which were subjected to acute uranium poisoning. Boston Med. and Surg. Jour., 1908, clix, 8; and The Archives Int. Med., 1911, viii, 469.

severe cases in which the majority of the glomeruli are extensively damaged, the few more normal appearing glomeruli are much larger than normal, often showing over twice the normal diameter, a condition undoubtedly due to a compensating hypertrophy.

There is very constantly a marked parenchymatous change in the tubular epithelium, although the extent of this depends largely on the time which has elapsed since the last injection of uranium. The convoluted tubules are, as a rule, less severely damaged than are the smaller tubules, but there is usually marked hydropic degeneration of the epithelium, and often a coarse, granular degeneration in which the granules take a deep eosin stain. Occasionally the convoluted tubules show pyknosis and desquamation of the epithelium, and contain much exudate and débris in their dilated lumens. A few tubules are widely dilated and form small cysts. The most severe and most constant parenchymatous degeneration is found in the ascending limbs of Henle, and in the smaller connecting tubules. Here there is marked pyknosis, desquamation, and often complete necrosis of the epithelium, and frequently almost complete blocking with desquamated cells and dense hyaline casts. In the few cases where fatty degeneration is present, it is almost wholly confined to the epithelium of the ascending limbs of Henle. In the areas of fibrosis many of the tubules are collapsed and shrunken, and in many places are represented by small accumulations of pyknotic and fragmented nuclei.

In the majority of cases the collecting tubules are quite normal in appearance excepting for the presence of exudate, desquamated cells and casts in their lumens, but in a few cases there is actual destruction and desquamation of the collecting tubule epithelium, and in some places there seems to be an attempt at regeneration of the epithelial covering.

In none of the cases is there any constant and characteristic change in the smaller blood-vessels. In practically all of the more fibrotic kidneys there is a definite peri-arterial fibrosis which seems to be more marked in dogs than in the other animals, but nowhere could be found changes in the intima and media of the smaller arteries such as are constantly present in chronic diffuse nephritis in man.

A fairly complete review of the literature on experimental chronic nephritis may be found in my previous report,[1] and also in reports made by Ophüls,[4] Lyon,[5] Pearce[6] and Christian,[7] so that a further review will not be made at this time.

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4. Ophüls: Experimental Chronic Nephritis. Jour. Am. Med. Assn., 1907, xlviii, 483.

5. Lyon: Experimental Study on the Action of Some Poisons and Toxins on the Kidney. Jour. Path. and Bact., 1903-04, ix, 400.

6. Pearce: Problems of experimental nephritis. The Archives Int. Med., 1910, v, 133.

7. Christian: Experimental Nephritis. Boston Med. and Surg. Jour., 1908, clviii, 416.

The method of experiment was the same as employed in my first series. The uranium nitrate was injected subcutaneously in an aqueous solution, and the number and frequency of the injections was controlled chiefly by the variations in the weight of the animals. Routine urine examinations were not made except in isolated cases, but from time to time specimens of urine from the dogs were collected and subjected to the usual clinical methods of examination.

The histological picture of acute uranium nephritis has been so frequently described that I shall not include in the present series any of the animals which succumbed during the first four or five weeks of the experiment.

A. FREQUENTLY REPEATED INJECTIONS OF SMALL DOSES

1. EXPERIMENTS ON GUINEA-PIGS

Protocol 1.—Guinea-pig 2, 1909, received twenty-six injections of 0.5 mg., and seven injections of 1 mg. of uranium nitrate during 217 days. The animal died after receiving the series of 1 mg. doses. Weight at beginning of experiment 750 gm. and at the time of death 650 gm.

Autopsy: No subcutaneous edema, no ascites; 5 c.c. of urine showed much albumin and sugar and a few granular casts; kidneys normal in size, pale, capsule slightly adherent, cut surface cloudy and pale, medulla darker than cortex; liver somewhat congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: The connective tissue proliferation is not very marked and is confined to the inner zone of the cortex. There are many areas of round cell infiltration in the cortex. There is very marked parenchymatous change, especially in the smaller tubules, with much pyknosis and desquamation, and many hyaline casts.

Protocol 2.—Guinea-pig 3, 1909, received eighty injections of 0.5 mg., and four injections of 1 mg. of uranium nitrate during 696 days. The animal died two days after the last injection of 1 mg. Weight at beginning of experiment 550 gm., maximum weight 860 gm., weight at time of death 510 gm.

Autopsy: No subcutaneous edema, ascites or hydrothorax; 5 c.c. of urine showed no albumin or casts; kidneys normal size, red, smooth except for a few small dimples which are more numerous on the right kidney; capsule not adherent, cut surface cloudy, markings fairly distinct; liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Moderate proliferation in the interstitial connective tissue, in the usual location; very little parenchymatous change; glomerular and tubular changes characteristic.

Protocol 3.—Guinea-pig 4, 1909, received seventy injections of 0.5 mg. of uranium nitrate during 590 days, and died on the 595th day. Weight at beginning of experiment 440 gm., maximum weight 790 gm., weight at time of death 720 gm.

Autopsy: No subcutaneous edema or urine; about 30 c.c. of slightly blood-stained fluid removed from peritoneal sac; kidneys small, surface pale with small hemorrhagic spots, capsule not adherent, cut surface somewhat cloudy; intestines much congested, liver pale, heart and aorta apparently normal.

Microscopical Examination: Moderate increase of interstitial connective tissue, almost entirely located in inner zone. Characteristic glomerular and tubular changes in the fibrotic areas. Little parenchymatous change in tubular epithelium. Many hyaline casts.

Protocol 4.—Guinea-pig 5, 1909, received eighty-six injections of 0.5 mg. of uranium nitrate during 695 days, and died six days after the last injection;

weight at the beginning of the experiment 520 gm., maximum weight 800 gm., weight at time of death 500 gm.

Autopsy: No subcutaneous edema, slight ascites and hydrothorax (10 c.c. of fluid collected from peritoneal sac and about 1 c.c. from each pleural sac). A few c.c. of urine showed a trace of albumin but no casts; kidneys about normal size, dark red, capsule definitely adherent, surface everywhere finely granular, cut surface bulges and is cloudy; no cysts seen; lungs and liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Very marked proliferation of interstitial connective tissue in both inner and outer zones of cortex; many rays extend to the surface; glomeruli show characteristic changes; some are cystic; tubular epithelium shows little degeneration except in fibrotic areas; many hyaline casts.

Protocol 5.—Guinea-pig 6, 1909, received forty-seven injections of 0.5 mg. of uranium nitrate during 360 days, and died on the 434th day. Weight at beginning of experiment 660 gm., maximum weight 770 gm., weight at time of death 640 gm.

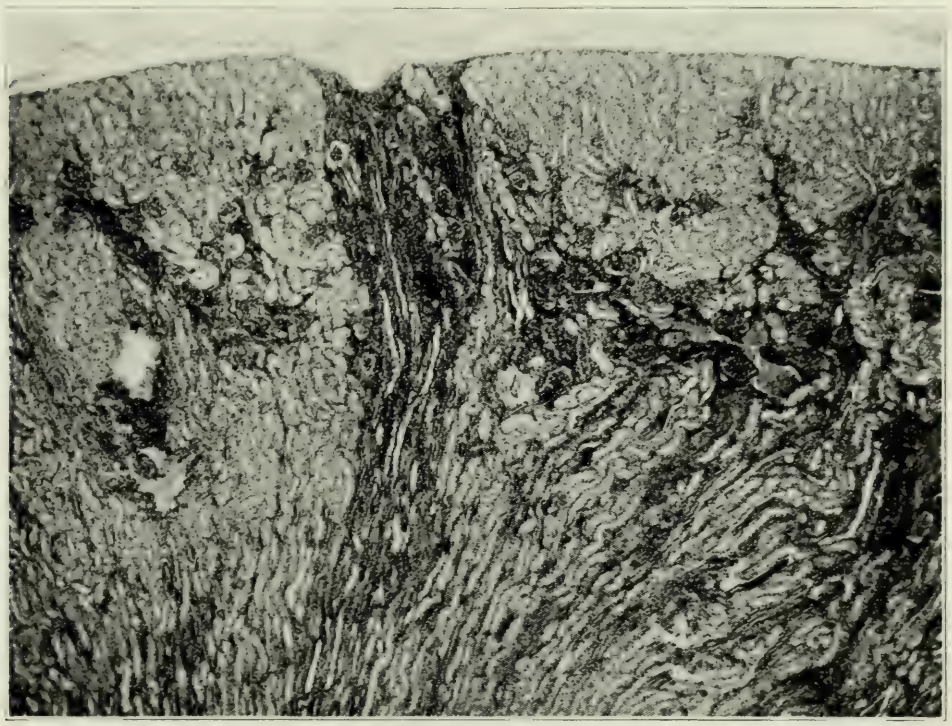

Fig. 1.—Guinea-Pig 14, Series B, Protocol 7. × 25. Note the wide distribution of the connective tissue proliferation in the inner zone of the cortex, and the rays extending toward the surface; also the depression at the place where one ray reaches the surface.

Autopsy: No subcutaneous edema and no urine; there was a small amount of fluid in the peritoneal sac and the stomach was distended with fluid; kidneys slightly smaller than normal, mottled, slightly dimpled, capsule not adherent, cut surface cloudy, not swollen; liver congested, heart and aorta apparently normal.

Microscopical Examination: Slight connective tissue proliferation in inner zone of cortex; some glomeruli show characteristic changes and a few are cystic; very marked parenchymatous change with much desquamation; many hyaline casts.

Protocol 6.—Guinea-pig 27, 1909, received thirty-seven injections of 0.5 mg. of uranium nitrate during 360 days, and died on the 429th day. Weight at beginning of experiment 570 gm., maximum weight 880 gm., weight at time of death 630 gm.

Autopsy: No subcutaneous edema and no urine; a small amount of fluid in peritoneal sac; kidneys normal size, capsule slightly adherent, surface dark red and finely granular, cut surface not cloudy, markings fairly distinct; liver and lungs congested, aorta apparently normal; right ventricle somewhat dilated.

Microscopical Examination: Moderate proliferation of interstitial connective tissue with many rays extending to the surface; glomeruli show usual changes, and some are cystic; there is moderate parenchymatous change with desquamation in the smaller tubules, and many hyaline casts; the tubules in the fibrotic areas are shrunken and collapsed.

Fig. 2.—Guinea-Pig 14. × 90. Note the proximity to a large blood vessel, the marked fibrosis, and the shrinkage of tubules in the fibrotic area.

II. EXPERIMENTS ON RABBITS

Protocol 1.—Rabbit 11, 1909, received thirty-two injections of 1 mg. of uranium nitrate during 196 days, and died on the 203d day. Weight at beginning of experiment 3,075 gm. and at the time of death 2,020 gm.

Autopsy: No subcutaneous edema, no ascites, and no urine. Double fibrino-purulent pleurisy. Kidneys somewhat enlarged, surface smooth, capsule not adherent, cut surface bulged and distinctly cloudy. Liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Marked diffuse proliferation of interstitial connective tissue, with many rays extending to the surface, and with many areas of round cell infiltration; glomeruli show thickening of capillary walls, and much

exudate in capsular spaces; marked parenchymatous degeneration of tubular epithelium, with much desquamation in Henle's loops and collecting tubules, and many hyaline casts; marked atrophy and collapse of tubules in the fibrotic areas.

Protocol 2.—Rabbit 14, 1909, received thirty subcutaneous injections of 1 mg. of uranium nitrate during 193 days, and died on the 196th day. Weight at beginning of experiment 3.250 gm., at time of death 2,430 gm.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys large, pale, smooth, with numerous small scars on surface, capsule not adherent; on section was some bulging and marked opacity; the cortex was pale and the medulla dark red; liver congested, right ventricle dilated, aorta apparently normal; marked double fibrino-purulent pleurisy.

Fig. 3.—Guinea-Pig 8, Series B, Protocol 1. × 100. Note the marked periarterial fibrosis, and the proximity of the damaged glomeruli to the vessel.

Microscopical Examination: Very marked, diffuse proliferation of interstitial connective tissue, with many large areas of round cell infiltration. The majority of the glomeruli and tubules are damaged; marked parenchymatous degeneration; much desquamation in smaller tubules and many hyaline casts.

Protocol 3.—Rabbit 15, 1909, received thirty-three subcutaneous injections of 1 mg. of uranium nitrate during 274 days, and was killed on the 457th day Weight at the beginning of the experiment 2.850 gm., at the time of death 2.950 gm.

Autopsy: No subcutaneous edema, slight ascites, no urine; kidneys rather small, pale and slightly granular; capsule not adherent; on the surface

were a few small hemorrhagic spots and a few small cysts. On section the cut surface was pale and the cortex was apparently thinner than normal.

Microscopical Examination: Very marked proliferation of interstitial connective tissue, with marked inner fibrosis, and many rays extending to surface; marked glomerular changes; capillary walls of tufts thickened, and many of the capsules cystic; moderate parenchymatous change, little desquamation, many tubules involved in fibrotic areas, shrunken; many hyaline casts.

Protocol 4.—Rabbit 18, 1909, received thirty-four injections of 1 mg. of uranium nitrate during 278 days, and died about thirteen months after the first injection.

Autopsy: Kidneys rather small, pale, finely granular, capsule not adherent; cut surface not swollen, cortex thinner than normal.

Microscopical Examination: Very marked and very diffuse proliferation of interstitial connective tissue, with many rays of cellular connective tissue extending outwards from the inner fibrotic area; very many areas of round cell infiltra-

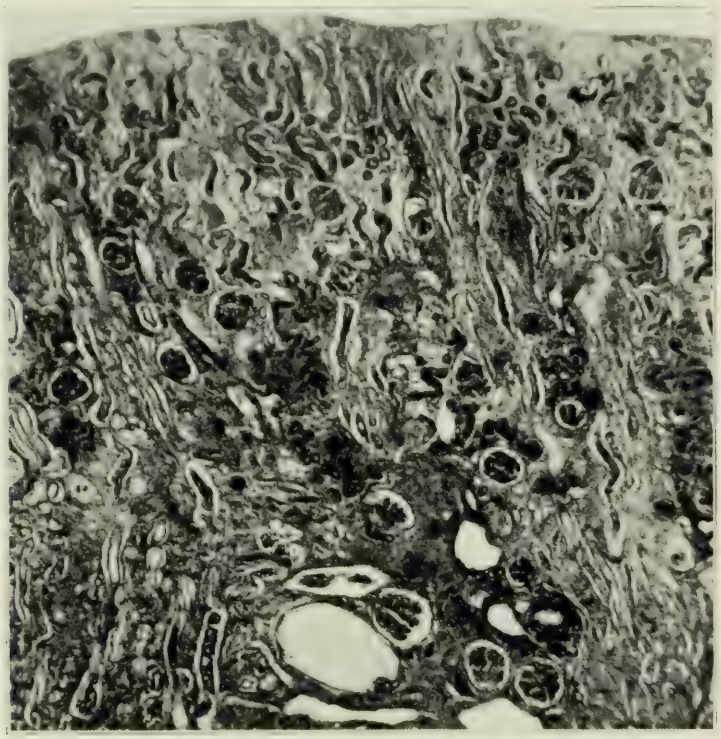

Fig. 4.—Rabbit 41, Series B, Protocol 12. × 40. Note the wide distribution of the lesion, the greater severity in the inner zone of the cortex, and the large glomerular cyst.

tion in the cortex; extensive glomerular changes; thickening of capillary walls, increase in connective tissue in tufts, and dilatation of capsular spaces; marked parenchymatous degeneration of tubules with much shrinkage and desquamation; marked desquamation of epithelium of collecting tubules; many hyaline casts; marked atrophy and collapse of tubules in the fibrotic areas.

B. OCCASIONAL SUBLETHAL DOSES

1. EXPERIMENTS ON GUINEA-PIGS

Protocol 1.—Guinea-pig 8, 1909, received ten injections of 5 mg. of uranium nitrate during 461 days, and died two days after the last injection; weight at beginning of experiment 620 gm., maximum weight 690 gm., weight at time of death 600 gm.

Autopsy: Very marked subcutaneous edema over belly and legs; no ascites, hydrothorax or urine; kidneys very small, about two-thirds normal size, surface pale, granular, capsule adherent, cut surface pale and cloudy, cortex thin; no cysts seen; liver and lungs congested, heart large, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Marked proliferation of interstitial connective tissue with many rays extending to the surface; little round cell infiltration; moderate characteristic changes in the glomeruli and tubules in the fibrotic areas; some glomerular cysts; relatively little parenchymatous change in the more normal appearing tubules; many hyaline casts.

Protocol 2.—Guinea-pig 9, 1909, received six injections of 5 mg. of uranium nitrate during 110 days, and died seven days after the last injection; weight at beginning of experiment 725 gm., at time of death 435 gm.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys slightly swollen, pale, slightly dimpled, capsule not adherent; cut surface cloudy and

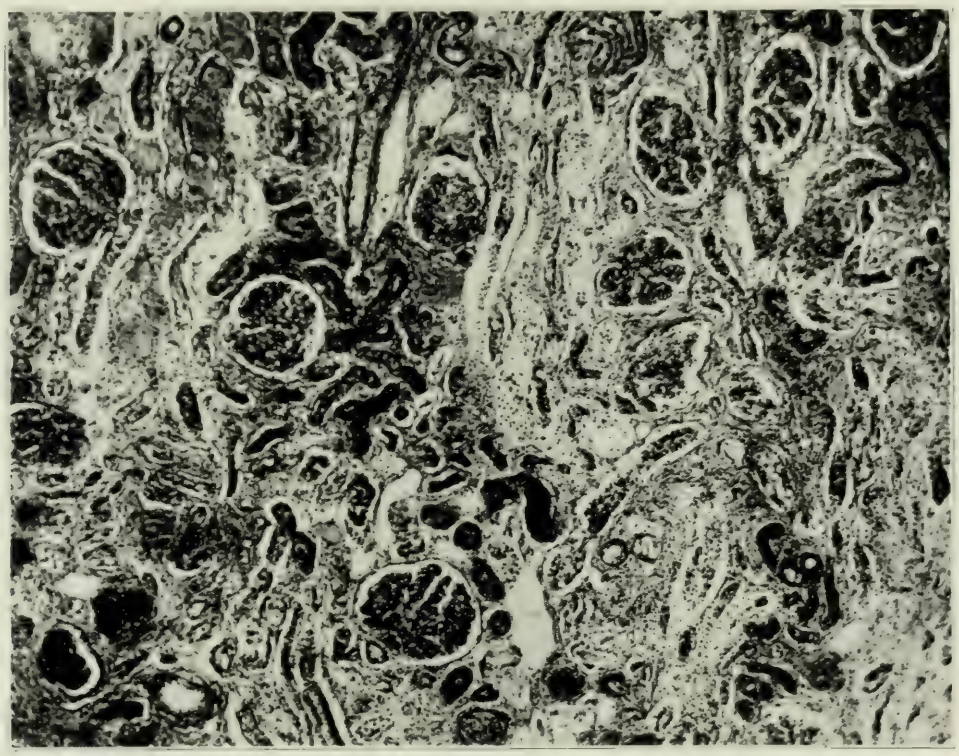

Fig. 5.—Rabbit 41. × 100. Note the diffuse distribution of the connective tissue proliferation, the collapse of the tubules and the pyknosis and desquamation of the tubular epithelium.

swollen, cortex paler than medulla; liver congested, right ventricle dilated; aorta apparently normal.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue which is very cellular; little shrinkage; marked glomerular changes with many cysts; marked parenchymatous change with much desquamation and many dense hyaline casts.

Protocol 3.—Guinea-pig 10, 1909, received eight injections of 5 mg. of uranium nitrate during 400 days, and died five days after the last injection; weight at beginning of experiment 520 gm., maximum weight 830 gm., weight at time of death 470 gm.

Autopsy: Moderate subcutaneous edema over belly, thorax and legs; 30 c.c. fluid removed from peritoneal sac, and several c.c. from both pleural sacs; peri-

cardium was distended with fluid to about twice normal size; no urine; kidneys about normal size, dark red, slightly dimpled, capsule slightly adherent, cut surface cloudy, not swollen; no cysts seen; liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Slight diffuse proliferation of interstitial connective tissue and many areas of round cell infiltration; relatively little glomerular change although a few capsules are cystic; very marked parenchymatous degeneration in all tubules, much desquamation and many hyaline casts.

Protocol 4.—Guinea-pig 11, 1909, received four injections of 5 mg. of uranium nitrate during 248 days, and died eleven days after the last injection; weight

Fig. 6.—Rabbit 50, Series B, Protocol 6. × 90. Note the dense fibrosis, the fibrous thickening of the glomerular capsules and the marked destruction of tubules in the fibrous area.

at beginning of experiment 590 gm., at time of last injection 730 gm., and at time of death 855 gm., an increase of 125 gm. during the last eleven days of life.

Autopsy: Very extreme subcutaneous edema over belly, thorax and legs; very marked ascites (110 c.c. collected and at least 30 c.c. escaped), a few drops of fluid in both pleural sacs, and moderate distention of pericardium; no urine; kidneys swollen, surface mottled, smooth, capsule not adherent, cut surface bulging, edematous and cloudy; liver congested and containing small focal necroses; moderate perihepatitis and perisplenitis; spleen large, right ventricle markedly dilated, aorta apparently normal.

Microscopical Examination: Some cellular proliferation of interstitial connective tissue, and a few areas of round cell infiltration; relatively little glomerular change; very marked parenchymatous change, much desquamation and a few hyaline casts.

Protocol 5.—Guinea-pig 12, 1909, received four injections of 5 gm. of uranium nitrate during seventy-five days, and died nine days after the last injection; weight at beginning of experiment 410 gm., maximum weight 480 gm., weight at time of death 295 gm.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys normal size, surface smooth with small dimples and a few pin point hemorrhages, capsule not

Fig. 7.—Rabbit 106, Series B, Protocol 11. × 90. Note the marked increase in fibrous tissue, the dense round cell infiltration, the thickening of the glomerular capsules, and the atrophy of the tubules.

adherent; cut surface swollen and cloudy; liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Slight cellular proliferation of connective tissue in inner zone of cortex and a few areas of round cell infiltration around some of the glomeruli; very marked parenchymatous change affecting all tubules, very numerous hyaline casts and a few cystic glomeruli.

Protocol 6.—Guinea-pig 13, 1909, received four injections of 5 mg. of uranium nitrate during seventy-seven days, and died three days after the last injection: weight at beginning of experime t 540 gm., weight at time of death 500 gm.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys normal size, surface shows few small dimples, capsule not adherent; cut section cloudy; a few small cysts; liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Slight cellular infiltration in inner zone of cortex with some areas of round cell infiltration in outer zone; little glomerular and tubular change; a few glomerular cysts and a few hyaline casts.

Protocol 7.—Guinea-pig 14, 1909, received seven injections of 5 mg. of uranium nitrate during 400 days, and died seven days after the last injection; weight at beginning of experiment 550 gm., maximum weight 680 gm., weight at time of death 310 gm.

Autopsy: No subcutaneous edema or hydrothorax; a few c.c. of ascitic fluid removed from peritoneal sac; 5 c.c. of urine showed a trace of albumin but no casts; kidneys small, pale, fibrous in appearance, surface granular, capsule adherent, cortex thin, cut surface slightly opaque. Liver and lungs congested, left ventricle seems thicker than normal, aorta apparently normal.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue and many large areas of round cell infiltration; very marked glomerular and tubular destruction in the fibrotic areas; little parenchymatous change in the more normal appearing tubules; many dense hyaline casts.

Fig. 8.—Dog 6, Series 1909, Protocol 5. Actual size. Note the marked dimpling of the surface, and the tears where the capsule was adherent.

Protocol 8.—Guinea-pig 17, 1909, received two injections of 10 mg. of uranium nitrate, with an interval of seventy-seven days, and died seven days after the last injection; weight at beginning of experiment 570 gm., weight ten days before death 650 gm., weight at time of death 380 gm.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys swollen, mottled, cloudy and smooth; capsule not adherent; liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Fairly diffuse cellular proliferation of interstitial connective tissue in inner zone of cortex; very marked parenchymatous degeneration with much desquamation and many hyaline casts; a few glomeruli contain exudate in their capsular spaces and some are dilated into small cysts.

Protocol 9.—Guinea-pig 18, 1909, received three injections, respectively, of 15, 10 and 5 mg. of uranium nitrate during 485 days, and died eleven days after the last injection; weight at beginning of experiment 800 gm., maximum weight 910 gm.; weight at time of death 540 gm.

Autopsy: No subcutaneous edema, 10 c.c. ascitic fluid and about 1 c.c. of fluid in each pleural sac and in pericardial sac; kidneys small, pale, granular with

adherent capsule, cortex thin, cut surface cloudy, no cysts seen; 10 c.c. urine showed heavy cloud of albumin but no casts.

Microscopical Examination: Moderate diffuse proliferation of interstitial cellular connective tissue, many areas of round cell infiltration and moderate fibrosis around the larger blood vessels; moderate glomerular change with many large cysts; moderate parenchymatous change, especially in the smaller tubules; many hyaline casts.

Protocol 10.—Guinea-pig 19, 1909, received two injections of 10 mg. of uranium nitrate with an interval of seventy-seven days, and died five days after the second injection.

Autopsy: No subcutaneous edema, ascites, or urine; kidneys mottled, smooth, with a few dimples, capsule not adherent, cortex cloudy; liver congested, heart and aorta apparently normal.

Microscopical Examination: Practically the same as described in Protocol 8.

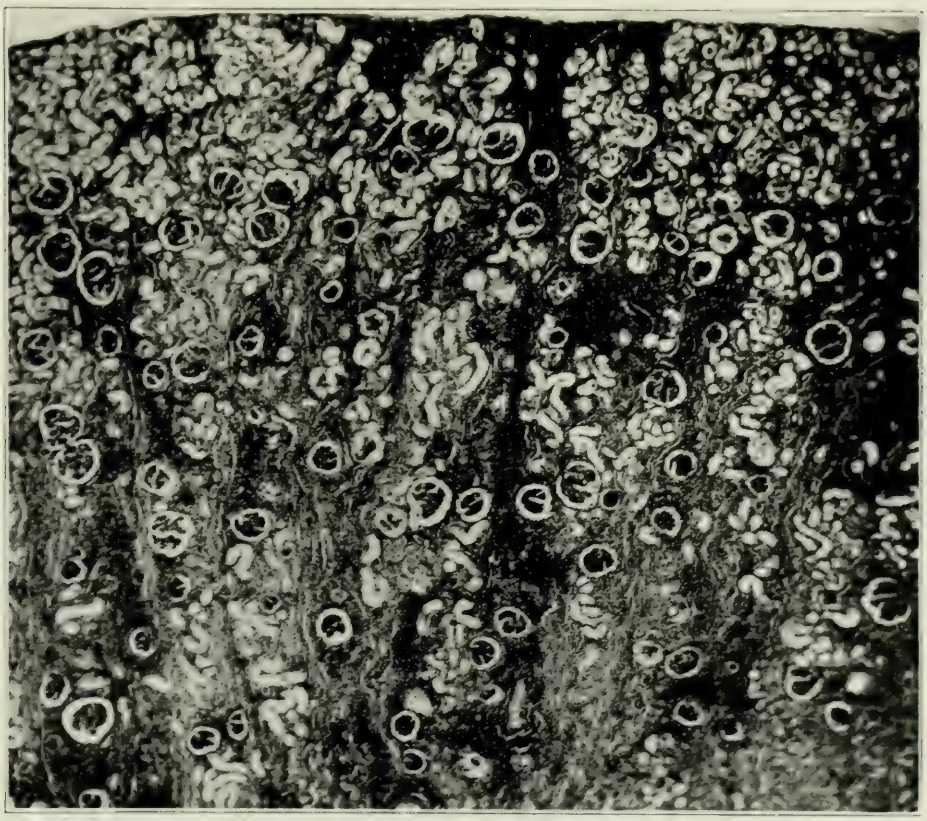

Fig. 9.—Dog 3, Series 1910, Protocol 8. × 25. Note the wide distribution and the severity of the lesions, the greater amount of connective tissue proliferation in the inner zone of the cortex, and the radiating rays extending toward the surface.

Protocol 11.—Guinea-pig 20, 1909, received three injections, respectively, of 10, 5 and 5 mg. of uranium nitrate during 485 days, and died five days after the last injection. Weight at beginning of experiment 610 gm., maximum weight 750 gm., weight at time of death 505 gm.

Autopsy: Moderate subcutaneous edema, moderate ascites and a few c.c. of urine which showed definite albumin and many granular casts; kidneys large, swollen, congested, capsule not adherent, cut surface swollen and cloudy; liver and lungs congested, right ventricle dilated, aorta normal.

Microscopical Examination: Irregularly scattered areas of fibrosis and diffuse proliferation of interstitial cellular connective tissue in cortex; marked cystic

dilatation of both tubules and glomeruli; the majority of the glomeruli show little damage; relatively little parenchymatous change; many hyaline casts.

B. EXPERIMENTS ON RABBITS

Protocol 1.—Rabbit 23, 1909, received 5 injections of 5 mg. of uranium nitrate during 111 days, and died on the 175th day.

Autopsy: No subcutaneous edema or ascites. Fifty c.c. of urine showed very marked albumin and many hyaline and granular casts. Kidneys swollen, dark red, surface smooth, capsule not adherent, cut surface cloudy. Liver congested, heart and aorta apparently normal.

Microscopical Examination: Marked proliferation of interstitial connective tissue. Marked glomerular change, with a hyaline deposit in some of the tufts. Many hypertrophied glomeruli. Marked destruction of tubules in fibrotic areas; relatively little parenchymatous change in more normal appearing tubules. Many hyaline casts.

Fig. 10.—Dog 3, Series 1910. × 100. Note the extreme fibrosis and round cell infiltration, the thickening and beginning cyst formation of the glomerular capsules, and the marked tubular destruction.

Protocol 2.—Rabbit 24, 1909, received four injections of 5 mg. of uranium nitrate during eighty-one days, and died seven days after the last injection.

Autopsy: No subcutaneous edema and no ascites; 10 c.c. of urine showed a moderate cloud of albumin and many granular casts; kidneys large, surface shows many small scars and some small hemorrhages, capsule slightly adherent, cut surface cloudy and swollen; liver congested, left ventricle seemed thicker than normal, aorta apparently normal.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue involving the whole cortex and outer part of the medulla; little fibrosis and little shrinkage; moderate glomerular change with some fibrosis and some hyaline deposit in the tufts; many of the tubules are separated by cellular proliferation of connective tissue; marked parenchymatous change, some desquamation, and many hyaline casts.

Protocol 3.—Rabbit 25, 1909, received three injections of 5 mg. of uranium nitrate during seventy-four days, and was killed on the 281st day; weight at beginning of experiment 2,200 gm., at the time of death 2,930 gm.

Autopsy: No subcutaneous edema or ascites; urine showed no albumin or casts; kidneys normal size, congested, capsule not adherent, surface smooth with few small scars, cut surface not swollen or cloudy; heart, liver and aorta normal.

Microscopical Examination: Scattered areas of cellular connective tissue proliferation in cortex and a few small areas of fibrosis in inner zone; majority of glomeruli and tubules normal; a few glomeruli have thickened capsules and pyknosis and fragmentation of nuclei, and a few tubules show hydropic and some granular degeneration.

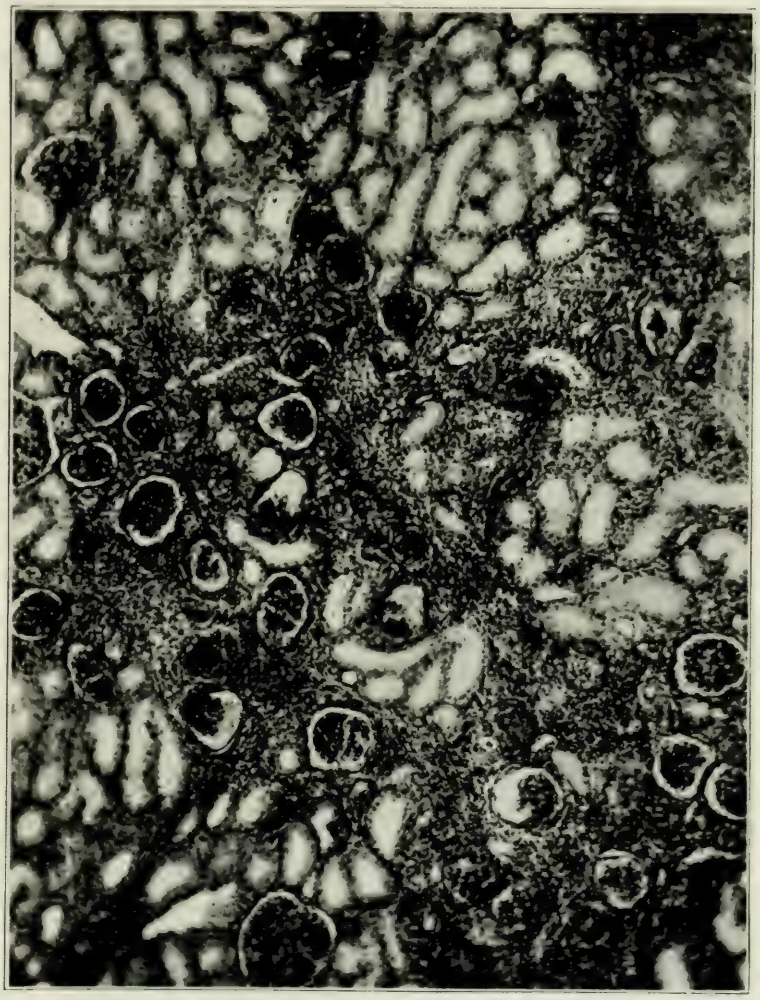

Fig. 11.—Dog 5, Series 1909, Protocol 4. × 90. Note the extreme degree of connective tissue proliferation, the cellular character of the newly-formed connective tissue, the marked shrinkage of the glomeruli, and the extensive tubular destruction.

Protocol 4.—Rabbit 26, 1909, received two injections of 5 mg. of uranium nitrate, with an interval of thirty-two days, and died seven days after the second injection.

Autopsy: No subcutaneous edema and no ascites; 25 c.c. urine showed a trace of albumin and many hyaline and epithelial casts; kidneys swollen, congested, surface smooth and mottled, capsule not adherent, cut section swollen and opaque; liver large and congested; heart and aorta normal.

Microscopical Examination: Very marked, diffuse, cellular proliferation of interstitial connective tissue in outer and inner zone of cortex; little fibrosis; many large areas of round cell infiltration in cortex and medulla; moderate glomerular and tubular change in fibrotic areas and marked parenchymatous degeneration with some desquamation and many hyaline casts.

Protocol 5.—Rabbit 27, 1909, received three injections of 5 mg. of uranium nitrate during seventy-four days and was killed on the 112th day. There had been a large abscess on the shoulder for about three weeks previous to death.

Autopsy: No subcutaneous edema, slight ascites and 30 c.c. of urine, which showed a trace of albumin but no casts; kidneys large, pale, smooth with a few dimples, capsule not adherent, cut surface swollen, cloudy and edematous; liver congested, heart and aorta apparently normal.

Fig. 12.—Dog 6, Series 1909, Protocol 5. × 60. Note the very marked periarterial fibrosis.

Microscopical Examination: Marked proliferation of interstitial connective tissue, with many areas of round cell infiltration in cortex; very marked glomerular and tubular change, especially in inner zone of cortex; many large glomerular cysts; marked parenchymatous change in tubules; much desquamation and many hyaline casts.

Protocol 6.—Rabbit 50, 1910, received one injection of 5 mg., and two injections of 3 mg. of uranium nitrate during 158 days, and died on the 264th day. Weight at beginning of experiment 1,680 gm., maximum weight 2,460 gm., weight at time of death 1,175 gm.

Autopsy: No subcutaneous edema, a few c.c. of ascitic fluid, and 15 c.c. urine, which showed a trace of albumin, many hyaline and waxy casts, and a few granular casts; kidneys small, pale, capsule not adherent, surface finely granular, cut section opaque, cortex thin, medulla edematous; liver congested, right ventricle dilated, left ventricle distinctly thicker than normal, aorta apparently normal.

Microscopical Examination: Marked diffuse proliferation of interstitial connective tissue. Marked glomerular change in inner zone of cortex, and some compensatory hypertrophy of glomeruli in outer zone; moderate parenchymatous change, some desquamation, and a few hyaline casts.

Protocol 7.—Rabbit 61, 1910, received three injections of 3 mg. of uranium nitrate during 150 days, and died on the 190th day. Weight at beginning of experiment 1,680 gm., maximum weight 1,810 gm., weight at time of death 1,390 gm.

Autopsy: No subcutaneous edema and no ascites; 10 c.c. of urine showed a faint trace of albumin but no casts; kidneys small, pale, finely granular, capsule not adherent, cut surface opaque; lungs and liver congested; heart dilated, aorta apparently normal.

Microscopical Examination: Moderate diffuse proliferation of interstitial connective tissue; relatively little glomerular change; marked parenchymatous change, especially in smaller tubules; many hyaline casts.

Protocol 8.—Guinea-pig 63, 1910, received three injections of 3 mg., and one injection of 2 mg. of uranium nitrate during 202 days, and died on the 242d day. Weight at beginning of experiment 1,730 gm., maximum weight 2,210 gm., weight at time of death 1,860 gm.

Autopsy: No subcutaneous edema, marked adhesive peritonitis with old adhesions between all viscera; 10 c.c. of urine showed definite cloud of albumin but no casts; kidneys normal size, pale, mottled, finely granular, capsule not adherent, cut surface opaque; liver and lungs congested, right ventricle widely dilated, left ventricle in extreme systole, hard, and apparently definitely hypertrophied, aorta normal.

Microscopical Examination: Moderate diffuse proliferation of interstitial connective tissue, with many areas of round cell infiltration scattered irregularly through the cortex; moderate glomerular change, with some compensatory hypertrophy of glomeruli in the outer zone; moderate parenchymatous change, and many dense hyaline casts.

Protocol 9.—Rabbit 67, 1910, received three injections of 3 mg. of uranium nitrate during 150 days, and died on the 175th day; weight at beginning of experiment 1,720 gm., maximum weight 2,010 gm., weight at time of death 1,250 gm.

Autopsy: No subcutaneous edema, ascites or hydrothorax; 20 c.c. urine showed a trace of albumin but no casts; kidneys small, slightly congested, surface smooth with a few dimples, capsule not adherent, cut surface cloudy, some edema in pyramids; liver and lungs congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Moderate diffuse proliferation of interstitial connective tissue which is much more cellular than usual; moderate glomerular change especially in inner zone; moderate parenchymatous change with desquamation, and many hyaline casts.

Protocol 10.—Rabbit 68, 1910, received three injections of 3 mg. of uranium nitrate during 150 days, and died six days after the last injection; weight at beginning of experiment 1,580 gm., maximum weight 1,780 gm., weight at time of death 1,200 gm.

Autopsy: No subcutaneous edema or ascites; a few c.c. of urine showed a marked cloud of albumin, many hyaline and a few granular casts; kidneys about normal size, capsule not adherent, surface dark red in color and everywhere covered by many large scars, cut surface cloudy.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue, and many areas of round cell infiltration in cortex; fairly

marked glomerular changes; marked parenchymatous degeneration of tubular epithelium; many hyaline casts.

Protocol 11.—Rabbit 106, 1910, received two injections of 3 mg. of uranium nitrate with an interval of 105 days, and died on the 127th day. Weight at beginning of experiment 2,260 gm., at time of death 1,200 gm.

Autopsy: No subcutaneous edema or ascites; right sided pleuropneumonia. A few c.c. of urine showed a trace of albumin and some hyaline casts; kidneys small, capsule adherent, surface pale and finely granular, cut surface pale, cortex thin; liver congested, right ventricle dilated, aorta apparently normal.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue with many rays extending to surface, and much diffuse proliferation of connective tissue between the rays; moderate glomerular change, and marked parenchymatous degeneration of tubular epithelium with some desquamation and many hyaline casts.

Protocol 12.—Rabbit 41, 1910, received five injections of 3 mg. and one injection of 6 mg. of uranium nitrate during seventy-two and one-half weeks, and died thirty-six days after the last injection. Weight at beginning of experiment 1,660 gm., maximum weight 2,720 gm., weight at time of last injection 2,550 gm., and weight at time of death 1,770 gm.

Autopsy: No subcutaneous edema; moderate ascites, hydrothorax and hydropericardium; 20 c.c. urine showed much albumin and a few hyaline casts; kidneys large, mottled, capsule not adherent, surface finely granular, cut surface pale, cortex distinctly narrow, much edema in medulla; liver large, firm, rough with evidence of passive congestion and increase in connective tissue; heart large, left ventricle evidently hypertrophied, right ventricle dilated; lungs congested, adrenals large, spleen and aorta apparently normal; chronic adhesive peritonitis.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue with many rays extending to the surface, much proliferation in areas of cortex between the rays, and much round cell infiltration; moderate glomerular change, much pyknosis and fragmentation of nuclei of tufts, little thickening of capsule or proliferation of capsular epithelium; some exudate into capsular spaces and moderate cyst formation; very marked parenchymatous degeneration with complete necrosis in many tubules and much desquamation; evidence of epithelial regeneration in many tubules; few hyaline casts; marked periarterial fibrosis around many of medium sized and larger arteries.

EXPERIMENTS ON DOGS

The injections on dogs during the earlier experiments were made somewhat at random in order to ascertain the degree of tolerance of these animals for the drug, as well as the effects which would be produced on the kidneys. At times the injections were repeated at frequent, and at other times, at much longer intervals. It was found that the best results were obtained either when fairly large amounts of the drug were injected during as short a period of time as the animal could stand, or, when almost sublethal doses were given, at longer intervals. The maximum dose which can be used with safety is approximately 3 mg. per kilogram of weight. This amount produces a fairly acute attack of nephritis from which the animal usually recovers, and can often be safely repeated several times at intervals of several weeks. In every case, however, the initial dose should be somewhat smaller than this, and subsequent treatment should be based on the reaction which the animal shows to the first injection.

Protocol 1.—Dog 2, 1909, a female fox terrier about 5 months old, received two injections of 5 mg. and one injection of 10 mg. of uranium nitrate during fifty days, and was killed thirty-three weeks after the first injection. The weight at time of death was 5 kilos. The urine several times showed albumin and some casts.

Autopsy: No edema, no ascites; urine free from albumin or casts; kidneys small, slightly mottled, capsules slightly adherent, cut surface opaque but not swollen; heart, liver, lungs and aorta apparently normal.

Microscopical Examination: Little increase in interstitial connective tissue except around the larger blood vessels; several large areas of round cell infiltration in cortex around blood vessels and glomeruli; some glomerular change in inner zone of cortex; some parenchymatous change, more marked in tubules in region of round cell infiltration; no casts seen.

Protocol 2.—Dog 3, 1909, a mongrel male about 5 months old, received two injections of 5 mg. and one of 10 mg. of uranium nitrate during fifty days, and was killed thirty-four weeks after the first injection. Albumin and a few hyaline casts were found once during the course of the experiment. Weight at time of death 10.26 kilos.

Autopsy: No edema and no ascites; urine showed many hyaline and granular casts, but no albumin; kidneys about normal size, slightly mottled, capsule slightly adherent, cut surface cloudy; liver, lungs and aorta apparently normal; heart large and flabby, weight 80 gm., and the heart to body ratio 0.00779.

Microscopical Examination: No diffuse proliferation of interstitial connective tissue, but many foci of cellular proliferation are scattered irregularly through the cortex; some glomerular change, especially near the areas of newly-formed connective tissue. In the inner zone a few glomeruli show almost complete fibrosis of the tufts, and some are cystic; relatively little parenchymatous change; some exudate and desquamation and a few hyaline casts.

Protocol 3.—Dog 4, 1909, a mongrel male about 5 months old, received one injection of 10 mg., and one of 20 mg. of uranium nitrate within seventeen days, and was killed thirty-one weeks after the first injection. Weight at time of death 14.28 kilos. The urine showed albumin and casts several times during the course of the experiment.

Autopsy: No edema or ascites; urine showed a few hyaline casts but no albumin; kidneys slightly larger than normal, mottled, capsule not adherent, surface slightly dimpled, cut surface cloudy and edematous, pyramids more congested than the cortex; liver and lungs congested, aorta apparently normal; heart large, left ventricle apparently hypertrophied, right ventricle dilated, weight 118 gm., and heart to body ratio 0.00802.

Microscopical Examination: Little new formation of fibrous tissue except around the larger blood vessels; several large areas of cellular infiltration in cortex where round cells and fibroblasts widely separate the kidney tubules; little glomerular change except in the areas of cellular proliferation; very marked parenchymatous change in practically all tubules, much pyknosis and desquamation in the smaller tubules, and many dense, hyaline casts.

Protocol 4.—Dog 5, 1909, a mongrel male about 5 months old, received two injections of 10 mg. of uranium nitrate during seventeen days, and was found dead fourteen weeks after the first injection. Weight at time of death 5 kilos.

Autopsy: No edema, ascites or urine; kidneys small, pale with minute hemorrhages, capsule thick and firmly adherent, surface torn and rough, with numerous large and smaller depressions; no cysts seen; cut surface white and fibrous, markings almost obliterated, cortex very narow; liver large and congested; heart large, left ventricle firm and appears hypertrophied; aorta apparently normal; weight of heart not obtained.

Microscopical Examination: Very marked proliferation of interstitial connective tissue in both cortex and medulla, and marked fibrous thickening of the capsule; newly-formed connective tissue very fibrous in inner zone of cortex

from which more cellular rays extend to surface; marked, diffuse connective tissue proliferation in areas of cortex between the rays; practically all glomeruli extensively involved; much fibrosis of tufts and thickening of capsules, but no cyst formation. A few glomeruli show compensatory hypertrophy; very extensive tubular destruction with much desquamation in smaller tubules, and extreme atrophy of tubules in the fibrotic areas; much more fibrosis in medulla than usual; no casts seen.

Protocol 5.—Dog 6, 1909, a mongrel male about 5 months old, received two injections of 10 mg. of uranium nitrate during seventeen days, and was killed twenty-nine weeks after the first injection. Albumin but no casts were found twice during life. Weight at time of death 5.61 kilos.

Autopsy: No subcutaneous edema and no ascites; 30 c.c. urine showed many granular and hyaline casts but no albumin; kidneys small, pale, capsules firmly adherent, surface torn, finely granular, with some large scars and a few small cysts; cut surface very pale and fibrous, cortex thin, medulla white and glistening; liver large and congested; aorta apparently normal; heart large, left ventricle thick and firm, right ventricle dilated, weight 60 gm.; heart to body ratio 0.01069.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue, more marked and more fibrous in inner zone of cortex, with more cellular rays extending to the surface; much diffuse proliferation of connective tissue in cortex between the rays, and many areas of round cell infiltration; very marked change in practically all glomeruli, much shrinkage and capsular thickening, and many small cysts; some glomeruli show compensatory hypertrophy; practically none of the tubules normal, much dilatation of lumens, little desquamation of epithelium but much exudate; much increase in fibrous tissue in medulla; very few casts seen.

Protocol 6.—Dog 1, 1910, a mongrel female about 9 months old, received four injections of 10 mg. and three injections of 20 mg. of uranium nitrate during forty-five weeks, and was accidentally killed sixty-one weeks after the first injection. Weight at beginning of experiment 7.5 kilos, weight at time of death 7.68 kilos.

Autopsy: No subcutaneous edema or ascites, no urine; kidneys small, reddish brown in color, capsule slightly adherent, surface slightly granular, cut surface apparently normal; liver, lungs and aorta normal; heart rather large, in diastole, weight 61.5 gm.; heart to body ratio 0.00800.

Microscopical Examination: Marked proliferation of interstitial connective tissue, quite cellular in outer zone; much round cell infiltration; moderate glomerular change; marked parenchymatous change; much fat, especially in ascending limbs of Henle; little desquamation; much shrinkage in areas of fibrosis; few hyaline casts.

Protocol 7.—Dog 2, 1910, a male spaniel, about 6 months old, received three injections of 5 mg., one injection of 10 mg., and two injections of 20 mg. of uranium nitrate during fifty-three weeks, and was killed seventy-one weeks after the first injection. Weight at beginning of experiment 5.25 kilos, weight at time of death 16.3 kilos.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys about normal size, dark red, capsule slightly adherent, surface smooth, cut surface opaque and slightly edematous; liver, lungs, adrenals and aorta apparently normal; heart large, right side dilated, weight 145 gm., heart to body ratio 0.00888.

Microscopical Examination: Marked diffuse proliferation of interstitial connective tissue which is quite cellular, and many large areas of round cell infiltration; marked change in many of the glomeruli, with lobulation and shrinkage of tufts and thickening of capsules; in some glomerular spaces there are masses of epithelium which are evidently pushed in from the tubules; a few glomeruli show compensatory hypertrophy; very marked parenchymatous change in all tubules, marked desquamation in smaller tubules, a few casts.

Protocol 8.—Dog 3, 1910, a mongrel male, about 9 months old, received two injections of 10 mg., two of 30 mg., and three of 50 mg. of uranium nitrate during fifty-three weeks, and was killed sixty weeks after the first injection; weight at beginning of experiment 14.2 kilos, weight at time of death 21.6 kilos; urine examinations during life showed traces of albumin and a few hyaline casts.

Autopsy: No subcutaneous edema, no ascites, no urine; kidneys normal in size, brownish in color, capsule slightly adherent, surface fairly smooth, cut surface cloudy, cortex slightly narrower than normal; liver, pancreas and aorta apparently normal; heart large, left ventricle firm and thick, weight 180 gm.; heart to body ratio 0.00818.

Microscopical Examination: Very marked diffuse proliferation of interstitial connective tissue which in most places does not reach the surface; many areas of round cell infiltration; marked glomerular change, many tufts shrunken, distinct fibrosis of some tufts, much exudate in spaces and marked thickening of capsules; many glomeruli are hypertrophied; very marked parenchymatous change in all tubules, much desquamation, much fatty degeneration in convoluted tubules and ascending limbs of Henle; no casts seen.

Protocol 9.—Dog 5, 1910, a black and tan male about 9 months old, received three injections of 10 mg. and one of 30 mg. of uranium nitrate during twenty-three weeks, and died eleven days after the last injection. Weight at beginning of experiment 8.98 kilos, and at time of death 8.02 kilos.

Autopsy: No subcutaneous edema and no ascites; 30 c.c. of urine in bladder showed much albumin but no casts; kidneys firm, swollen, congested, capsule slightly adherent, surface smooth, cut surface swollen and cloudy; liver, lungs and aorta apparently normal; heart large, right ventricle dilated, left ventricle apparently enlarged, weight 73 gm.

Microscopical Examination: Moderate increase in interstitial connective tissue, many areas of round cell infiltration; very little glomerular change; marked parenchymatous change with some desquamation and many hyaline casts.

Protocol 10.—Dog 7, 1910, a mongrel male about 8 months old, received three injections of 10 mg., two of 30 mg. and three of 40 mg. of uranium nitrate during fifty-three weeks, and died thirteen days after the last injection; weight at beginning of experiment 8.9 kilos, weight at time of last injection 12.9 kilos, and weight at time of death 10.5 kilos.

Autopsy: No subcutaneous edema, no ascites; 3 c.c. urine showed much albumin and a few granular casts; kidneys about normal size, very pale, capsule slightly adherent, surface distinctly granular with fine and coarser nodules; cut surface pale and very opaque, cortex thin; liver and lungs congested, aorta apparently normal; heart large and flabby, many subendocardial hemorrhages; all chambers dilated.

Microscopical Examination: Moderate, diffuse, cellular proliferation of interstitial connective tissue with very marked periarterial fibrosis; moderate glomerular change; very extreme parenchymatous change with almost complete loss of nuclear staining; very marked desquamation, much exudate, and many hyaline casts.

Protocol 11.—Dog 11, 1910, a mongrel male, received two injections of 50 mg. and three injections of 30 mg. during forty weeks, and died eight days after the last injection; weight at beginning of experiment about 12 kilos, weight at time of death 9.9 kilos. Daily routine urine examinations after each of the first three injections showed the characteristic urinary findings of acute uranium poisoning, with much albumin and sugar, and many granular casts.

Autopsy: No subcutaneous edema or ascites; 10 c.c. urine showed much albumin and sugar, and many granular and cellular casts; kidneys rather small, pale, capsule slightly adherent, surface slightly granular, cut surface cloudy; cortex narrow; liver and lungs congested, aorta apparently normal; heart dilated in all chambers; weight 76 gm.

Microscopical Examination: Moderate diffuse proliferation of cellular interstitial connective tissue with little fibrosis excepting around the larger vessels. Moderate glomerular change; no glomerular cysts; very marked parenchymatous change, much desquamation in smaller tubules, much exudate and many casts.

Protocol 12.—Dog 12, 1910, a young terrier female, received one injection each of 30, 50 and 40 mg. of uranium nitrate during twelve weeks, and died four weeks after the last injection. Weight at beginning of experiment 10.5 kilos, maximum weight 15.8 kilos, weight at time of death 12.9 kilos.

Autopsy: No subcutaneous edema, ascites or urine; kidneys large, dark red, capsule slightly adherent, surface slightly granular, cut surface moist and cloudy, no cysts seen; liver and lungs congested, aorta apparently normal; heart dilated, numerous subendocardial ecchymoses in left ventricle; weight 85 gm.

Microscopical Examination: Moderate, diffuse and very cellular proliferation of interstitial connective tissue, with many large areas of round cell infiltration; relatively little glomerular change; few glomerular cysts; marked parenchymatous change, much flattening of tubular epithelium, some desquamation and much exudate; few casts seen.

Protocol 13.—Dog 15, 1910, a fox terrier female about 8 months old, received one injection each of 15 mg. and 20 mg. of uranium nitrate with an interval of nine weeks, and died eleven days after the last injection. Weight at beginning of experiment 5.3 kilos; weight at time of last injection 5.2 kilos, weight at time of death 3.6 kilos.

Autopsy: No subcutaneous edema and no ascites; 15 c.c. urine showed much albumin, moderate glycosuria, very many granular, and many waxy casts; kidneys swollen, congested, capsule not adherent, surface smooth, cut surface bulges, cortex wide and opaque; liver and lungs congested, aorta apparently normal; heart small, right ventricle dilated.

Microscopical Examination: Slight diffuse cellular proliferation of interstitial connective tissue; many areas of round cell infiltration; little glomerular change; some parenchymatous degeneration with little desquamation; no casts seen.

Protocol 14.—Dog 17, 1910, a fox terrier female about 8 months old, received two injections of 20 mg. of uranium nitrate with an interval of nine weeks, and died 11 days after the last injection. Weight at beginning of experiment 7.7 kilos, weight at time of death 5 kilos.

Autopsy: No subcutaneous edema and no ascites; 5 c.c. urine showed much albumin, some sugar, very many granular, and a few cellular casts; kidneys large, swollen, congested, capsule not adherent, surface smooth, cut surface bulges and is opaque; liver and lungs congested, aorta apparently normal; heart large, right side dilated, weight 50 gm.

Microscopical Examination: Slight, diffuse, cellular proliferation of interstitial connective tissue with rays extending almost to periphery in places; glomeruli show some exudate but little chronic change; very marked parenchymatous change, almost complete necrosis of epithelium, much desquamation and many casts.

Protocol 15.—Dog 19, 1910, a fox terrier female about 6 months old, received six injections of 10 mg. of uranium nitrate during eleven weeks, the first five being given during the first five weeks, and died five weeks after the last injection. Weight at beginning of experiment 3.7 kilos, weight at time of last injection 4.7 kilos, weight at time of death 2.5 kilos.

Autopsy: No subcutaneous edema and no ascites; 10 c.c. urine showed much albumin and sugar and very many granular and hyaline casts; kidneys small, pale, firm, capsule adherent, surface roughened, cut surface pale and opaque; liver, lungs and aorta apparently normal; heart small, not dilated, weight 26 gm.

Microscopical Examination: Slight cellular proliferation of interstitial connective tissue in inner zone of cortex with rays extending outwards to the surface; very little glomerular change; very marked parenchymatous change with almost complete necrosis of epithelium in many tubules; few casts.

Protocol 16.—Dog 22, 1910, a fox terrier female about 6 months old, received six injections of 5 mg. of uranium nitrate during eleven weeks, and died twenty-five weeks after the first injection; weight at beginning of experiment 4.4 kilos; weight at time of last injection 5.6 kilos; weight at time of death 4.3 kilos.

Autopsy: No subcutaneous edema and no ascites; 15 c.c. urine showed much albumin, some sugar and very many hyaline and partly granular casts; kidneys small, pale, capsule slightly adherent; surface smooth; cut surface cloudy and bulging; medulla darker than cortex; liver and lungs congested, aorta apparently normal; heart small, not dilated, weight 38 gm.

Microscopical Examination: Very marked, fairly diffuse cellular proliferation of interstitial connective tissue which in most places does not reach to the surface; moderate glomerular change, some exudate and some cysts; very marked parenchymatous change, marked fatty degeneration especially in Henle's loops; little desquamation and no casts.

Protocol 17.—Dog 24, 1910, a mongrel female about 6 months old, received seven injections of 5 mg. of uranium nitrate during thirty-one weeks, the first five being given during the first five weeks, and died nine days after the last injection. Weight at beginning of experiment 5.6 kilos, weight at time of last injection 6.9 kilos, weight at time of death 5.2 kilos.

Autopsy: No subcutaneous edema, ascites or urine; kidneys small, pale, capsule slightly adherent, cut surface cloudy and apparently more fibrous than normal; liver and lungs congested, aorta apparently normal; heart not dilated; weight 55 gm.

Microscopical Examination: Moderate, diffuse, cellular proliferation of interstitial connective tissue which does not quite reach the surface in most places; capsule moderately thickened; relatively little glomerular change, some exudate but no cysts; marked parenchymatous change, little desquamation, much exudate, and many dense, hyaline casts.

Protocol 18.—Dog 26, 1910, a young pointer, received two injections of 50 mg. of uranium nitrate with an interval of six and one-half weeks, and died seventeen weeks after the first injection; weight at beginning of experiment 20.5 kilos, weight at time of second injection 20.3 kilos, weight at time of death 12 kilos.

Autopsy: No subcutaneous edema or ascites. Five c.c. urine showed a trace of albumin but no casts; kidneys normal size, pale, capsule slightly adherent, surface smooth, cut surface cloudy, cortex appears rather reduced; liver, lungs and aorta normal; heart large, many subendocardial ecchymoses, no dilatation, weight 113 gm.

Microscopical Examination: Very marked cellular proliferation of interstitial connective tissue which is very diffusely distributed; kidney capsule markedly thickened; moderate glomerular change, much exudate and some small cysts; marked parenchymatous change, many tubules almost completely necrotic; moderate desquamation, much exudate and many dense hyaline casts. In places there is cystic dilatation of some of the tubules.

Protocol 19.—Dog 27, 1910, a young mongrel male, received two injections of 40 mg. of uranium nitrate with an interval of six and one-half weeks, and died twenty-four weeks after the first injection; weight at beginning of experiment 13.8 kilos, weight at time of second injection 16.7 kilos, weight at time of death 12 kilos.

Autopsy: No subcutaneous edema and no ascites; 15 c.c. urine showed much albumin and sugar and many granular casts; kidneys swollen and pale; capsule thick and firmly adherent, surface torn and finely granular with some coarser nodules, cut surface opaque, cortex thin with evident strands of connective tissue extending outwards to the surface; liver cloudy, lungs congested, aorta apparently normal; heart large, right ventricle dilated.

Microscopical Examination: Very extreme proliferation of interstitial connective tissue which involves practically the whole of the cortex, and is everywhere very cellular; very marked glomerular change, practically all glomeruli affected,

much exudate, proliferation and desquamation of capsular epithelium, and marked lobulation and shrinkage of tufts; a few glomeruli are hypertrophied; very extreme parenchymatous change in practically all tubules, with much dilatation of lumens, exudate, necrosis and desquamation; many tubules in areas of connective tissue proliferation are collapsed and atrophied; a few dense hyaline casts.

In discussing the conclusions which seem justifiable from the foregoing experiments, it will be more convenient to make a division under three headings: (1) edema formation; (2) renal changes; (3) cardiovascular changes.

EDEMA FORMATION

The use of uranium nitrate as an irritant with which to produce kidney lesions in animals was suggested by the reports of Richter,[8] in which he described the production of edema in rabbits by the administration of uranium salts. In my first series of experiments on guinea-pigs[1] varying amounts of ascites and hydrothorax were found in some cases, but in no instance was there any subcutaneous edema or general anasarca. Pearce[6] referred to this fact during a discussion of the experiments, and he suggested that more complete analogy to human nephritis might have been obtained if rabbits had been used for injection instead of guinea-pigs, since it had been shown that edema was so readily produced in rabbits during acute uranium intoxication, but the more recent experiments have shown that this is not the case.

In the series of sixteen experiments on rabbits which are here reported, but one animal showed any marked amount of fluid in the serous cavities, and only three others showed small amounts of ascites. In none of the cases was there any subcutaneous edema. It is true that three died of acute fibrino-purulent pleurisy, and that one showed a slight adhesive peritonitis, but these conditions were due to intercurrent infections and probably were in no way associated with any previous serous transudation.

The guinea-pig experiments showed vastly different results.[9] "In the complete series of seventeen animals which survived for longer than three months, seven which died before the eighth month showed no edema, and of the ten which lived for longer than eight months, nine showed definite, and in some cases, marked edema. In four of the nine cases there was marked subcutaneous edema, which in three cases was associated with effusion into the serous cavities." All of the cases of subcutaneous edema occurred in animals to which relatively large doses of the drug had been administered; and, with one exception, in every case in which edema was present, the animal died within eleven days after the last injection.

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8. Richter: Die experimentelle Erzeugung von Hydrops bei Nephritis. Beitr. z. klin. Med. Festschr. Herrn. Prof. Senator, Berlin, 1904, 283; and Experimentelle über der Nierenwassersucht, Berl. klin. Wchnschr., 1905, xlii, 384.

9. Dickson, E. C.: Edema Formation in Guinea-pigs in Chronic Experimental Uranium Nephritis. Proc. Soc. Exper. Biol. and Med., 1910, viii, 46.

The significance of these observations on guinea-pigs is at least twofold. In the first place, it is definitely proved that edema formation in animals resulting from the administration of uranium nitrate is not confined to rabbits, and in the second place, a study of the conditions under which the edema was produced will show that there has been established a much closer analogy to the edema formation of Bright's disease in man.

In the production of experimental uranium edema in rabbits by Richter,[8] Georgopulos,[10] Heinecke and Meyerstein[11] and others, the administration of uranium nitrate was always followed by forced water ingestion to produce an artificial hydremic plethora. In the experimental production of edema by combining the action of renal and vascular irritants, Pearce[12] also forced water ingestion, and he established as the three requisites for the production of renal edema, kidney damage, vascular damage and hydremic plethora. In my series of cases no attempt was made to induce edema by forcing water. Throughout the experiment the animals were kept in a large cage from which they were removed from time to time for weighing and for further injections. They were fed on a mixed diet in which was plenty of greens, and they obtained the whole of their water-supply from the green stuff which they consumed. A hydremic plethora due to increased water ingestion is obviously impossible under such conditions, and one can only assume that the increase in the fluids in the body must have been due to the suppression of urine which occurred during the terminal attack of acute nephritis.

The conclusion of all observers who have employed uranium salts for the experimental production of edema is that there must be some specific action of uranium in addition to its effect on the renal epithelium, which is at least partly responsible for the edema formation. The observations of Heinecke and Meyerstein,[11] who found that edema was produced in rabbits which were poisoned with chromates only after the serum of rabbits suffering from acute uranium poisoning had been injected intravenously, were very suggestive, even though the results were not constantly produced, and there seemed to be sufficient ground for their conclusion that there must be some vascular damage, in addition to the renal damage, which was responsible for the edema formation. The oncometer experiments of Schlayer and Hedinger[13] and Takayasu[14] gave

10. Georgopulos: Experimentelle Beitrage zur Frage des Nierenwassersucht. Ztschr. f. kin. Med., 1906, lx, 411.

11. Heinecke and Myerstein: Experimentelle Untersuchungen über die Hydrops bei Nierenkrankheiten. Deutsch. Arch. f. kin. Med., 1907, xc, 101.

12. Pearce: The Production of Edema; an Experimental Study of the Relative Etiologic Importance of Renal Injury, Vascular Injury and Plethoric Hydremia. The Archives Int. Med., 1909, iii, 422.

13. Schlayer and Hedinger: Experimentelle Studien über toxische Nephritis. Deutsch. Arch. f. klin. Med., 1907, xc, 1.

14. Schlayer, Hedinger and Takayasu: Ueber nephritisches Oedem. Deutsch. Arch. f. klin. Med., 1907, xci, 59.

the first experimental proof that such must indeed be the case, and their observations have been confirmed by Pearce and his assistants.[15] Pearce[12] has further established the importance of vascular injury in renal edema in the series of experiments to which I have already referred, namely, those in which he employed combinations of various vascular irritants with bichromate poisoning and forced water ingestion.

In view of these uniform conclusions as to the importance of damage to the blood-vessels in the production of renal edema, but one explanation can be given for its occurrence in the cases here described. The fact that the death of the animals and the onset of the edema occurred during the acute intoxication which followed the last injection of uranium, indicates definitely that the edema was a true renal edema, and not edema due to failing compensation of an overworked heart; and the appearance of the hearts at autopsy confirmed this view. There must therefore have been some condition present after the several months of treatment, which rendered the animals unable to cope with the hydremia of an acute attack of nephritis which was presumably no more severe than that of any one of the several previous attacks. It is difficult to see how this condition could be other than some damage to the smaller blood-vessels, especially in those cases in which the subcutaneous edema was so well developed. I believe we are justified in concluding that the repeated injections of uranium had so acted on the (blood) vascular system that it was no longer able to withstand the increase in the volume of blood which results from the partial or complete suppression of urine of acute uranium poisoning.

Whether or not this be the correct explanation, there can be no doubt that the formation of edema, occurring as it did in these animals during an attack of nephritis, and without any artificially produced hydremic plethora, establishes another step in the advance toward a complete analogy to Bright's disease in man.

RENAL CHANGES

The histological appearance of chronic uranium nephritis has already been described in the beginning of this report, and the characteristic type of the lesions, as well as the constancy with which they occur, has been mentioned. In order to determine whether similar lesions of spontaneous origin occur in normal animals, a series of routine histological examinations was made of the kidneys of fifty rabbits and 100 guinea-pigs which had been sacrificed for various purposes in the laboratory. The results of these observations have recently been reported by Ophüls,[16] from whom I shall quote the following:

15. Pearce, Hill and Eisenbrey: Experimental Acute Nephritis; the Vascular Relations and the Elimination of Nitrogen. Jour. Exper. Med., 1910, xii, No. 2, 196.

16. Ophüls, W.: Occurrence of Spontaneous Lesions in Kidneys and Livers of Rabbits and Guinea-Pigs. Proc. Soc. Exper. Biol. and Med., 1911, viii, 75.

In view of the importance of the occurrence of spontaneous lesions in the kidneys and in the livers of rabbits and of guinea-pigs in reference to the experimental work on these organs, Dr. E. C. Dickson and myself have made a careful study of these organs and incidentally of the heart and of the aorta in fifty rabbits and in one hundred guinea-pigs. The animals used were partly fresh animals from the market, partly animals raised at the laboratory. * * *

Twenty-eight rabbits, among them some of the old ones, had entirely normal kidneys, nine showed slight parenchymatous lesions, three a few small areas of cellular infiltration. In ten we found scattered small areas in which were marked interstitial lesions with the formation of small depressions on the surface. Four of these proved to be radially arranged chronic septic foci which extended from the vicinity of the papilla to the outer surface. In the other six cases the same arrangement of the newly-formed connective tissue in narrower radial bands which start deep in the pyramids was noticeable although no evidence of a septic infection could be discovered. The interstitial lesions were accompanied by marked epithelial lesions in two cases only, producing to a certain extent the picture of a chronic parenchymatous nephritis, although the lesions were never very extensive. Lesions of the blood-vessels or primary lesions of the glomeruli were not found in any case. * * *

Of the one hundred guinea-pigs the large majority had been injected with sediment from urine or other material suspected of containing tubercle bacilli; only a few actually developed tuberculosis. There were several especially selected old animals. Sixty-three of the one hundred had absolutely normal kidneys, two showed slight parenchymatous lesions and in thirty-five there were scattered small areas of cellular infiltration in the cortex which were sometimes rather numerous; at other times they were found with difficulty only on careful examination of several sections. They consist largely of lymphocytic cells and a few larger cells with vesicular nuclei. They do not seem to have much tendency to develop into fibrous tissue although occasionally such a change was observed but always to a limited degree only. * * * In few cases fibrous thickening of the capsules of glomeruli and cystic dilatation of the same were observed as spontaneous lesions similar to certain types of glomerular cysts described by Dickson in his paper on the "Experimental Production of Chronic Nephritis in Animals By the Use of Uranium Nitrate." These were, however, rare exceptions, whereas in uranium poisoning such glomerular lesions seem to be nearly constant. Large scars or lesions of the blood-vessels were not found in any case.

It will be seen from this extract that, although there was a fairly high percentage of cases which showed spontaneous kidney lesions, the type of the lesions which were found was entirely different from that of chronic uranium nephritis. Indeed, in some of the rabbits of my series there was quite evidently a combination of the two conditions, for in kidneys which were distinctly of the uranium type a few scattered foci could be recognized as more closely resembling the spontaneous type of lesions. And, moreover, although the spontaneous lesions which were found in the kidneys of rabbits were entirely different from those which were found in guinea-pigs, the lesions which were produced by uranium in both rabbits and guinea-pigs, and indeed also in dogs, were so similar as to be almost identical in many cases.

In the dog experiments, too, care was taken to exclude as much as possible the occurrence of spontaneous chronic kidney lesions. Young, healthy animals were always selected for experimentation, and of the nineteen cases here reported, fifteen were known to be less than 9 months

of age at the time of the first injection. In the 1909 series, Protocols 1 to 5,[17] especial precautions were taken to preclude the possibility of the development of spontaneous lesions. Puppies were weaned in the laboratory and were placed on a diet consisting of mush, bread and milk, with an occasional feeding of dried, chopped meat from which the extractives had been removed in the soup-pot. The animals thrived on this diet and received no other food during the whole course of the experiment. When they were 5 months old the series of injections of uranium nitrate were commenced, and, with the exception of one animal which was killed in a fight fourteen weeks after injection, they were allowed to live for from twenty-seven to thirty-four weeks after the first injection. The oldest of these puppies was 11½ months old when killed, so that in every case the experiment was terminated within the first year of life of the animal. The carefully regulated diet was such that the kidneys were protected as much as possible from the influence of any irritating substance excepting the uranium nitrate, and, when we consider in addition to this that in every case the animal was less than 1 year old at the end of the experiment, there seems little doubt that the lesions which were found must have been due to the administration of the uranium nitrate. Further experiments have confirmed this conclusion, for in the whole series of dogs which were observed during the following eighteen months, not one animal failed to show kidney lesions of a chronic nature, and of the uranium type, excepting those which died during the initial acute intoxication. The experiments show definitely that dogs are well adapted for the production of chronic kidney lesions, and that these lesions may be produced in practically all cases by the administration of uranium nitrate.

Although the occurrence of the chronic lesions in the kidneys has been so constant, the method by which these lesions are produced has not been revealed. A very striking feature of chronic uranium nephritis is that, even in the advanced cases in which the kidneys are hard and granular, and in which the greater part of the parenchyma is replaced by dense fibrous tissue, there is no demonstrable lesion in the vessels of the kidneys or of the peripheral vascular system. It is true that in many cases, especially in dogs, there is a peculiar peri-arterial development of fibrosis, and that in rabbits many of the glomerular tufts show almost complete fibrosis, but in the capillaries of these tufts the only evidence of lesion is a slight thickening of the walls, and the deposit of some hyaline substance around them, and in the larger arteries no lesions whatever can be demonstrated either in the intima or in the media. This fact is especially interesting in view of the recent experiments of Harvey[18] who, by the

17. The 1909 series of experiments on dogs was reported before the California Academy of Medicine in December, 1909. Cal. State Jour. Med., 1910, viii, No. 4, 140.

18. Harvey: Auto-intoxication and Experimental Nephritis in Rabbits. Jour. Path. and Bact., 1911, xvi, No. 1, 95.

administration of para-hydroxy-phenyl-ethylamine, was able to produce chronic lesions in the kidneys of rabbits secondarily to the development of atheroma of the aorta and arteriosclerosis of the renal artery and its branches. The kidneys which Harvey describes are undoubtedly of the arteriosclerotic type, and his experiments would seem to prove that the etiology of that type of nephritis is entirely different from that of such types of atrophied and granular kidneys as are represented by uranium chronic nephritis.

Another point of interest in these cases is one to which I referred in my previous report. In the kidney lesions of acute uranium poisoning the tubular epithelium is the part which shows the most marked anatomical change. With the exception of the formation of the hyaline bodies which Christian[3] has described in the glomerular tufts during acute uranium nephritis, no characteristic lesions have been found in the glomeruli, yet in the kidneys of chronic uranium nephritis the glomerular lesions are varied and severe. The most common, as well as the most extensive changes, are seen in the glomerular capsule, with its cystic dilatation, proliferated epithelium and hyaline basement membrane. The tufts also are more or less extensively damaged. In the less severe cases we find fragmentation of the nuclei and often some evidence of increase in connective tissue cells; and in the advanced cases there is distinct fibrosis and lobulation of the tufts, and, in rabbits, the thickening of the walls of the capillaries and the deposit of some hyaline substance in a part or the whole of the tufts, to which reference has already been made. Moreover, the compensatory hypertrophy of the more normal appearing glomeruli indicates diminution in function of the damaged glomeruli.

Still another interesting feature in chronic uranium nephritis is the characteristic distribution of the newly formed connective tissue. In every case it is more marked and much more fibrous in the inner zone of the cortex, and in the early cases it is in this region, especially around the larger blood-vessels, that the proliferation of connective tissue is first seen. Reference has already been made to the peculiar peri-arterial fibrosis which occurs around the larger arteries. From this deeper, more fibrous zone, the connective tissue extends outward toward the surface of the kidney, usually following the lines of the medullary rays. In those cases in which the surface of the kidney is granular, the processes extend to the surface and correspond to the small depressions on it, but in many cases where the surface is still quite smooth histological examination shows in the deeper parts much newly formed connective tissue from which many radiating processes extend into the outer zone of the cortex but do not reach the surface. In some kidneys there is also a diffuse connective tissue proliferation which more or less completely destroys the kidney cortex between the rays; but this seems to be a later stage in the process, and is found only in the most severe cases. Occasionally there is also a

more or less marked extension of the connective tissue proliferation into the medulla, but this is not at all constant.

A very striking feature of the connective tissue proliferation is that all the different stages are represented in the kidneys of animals which have not received any injections for several months. The condition is not that of a quiescent scar formation, for, although in the deeper portions the newly formed connective tissue is densely fibrous and contains but few nuclei, in the peripheral portions it is usually very cellular, with many spindle shaped cells with large, clear vesicular nuclei, and with many round plasma cells, clearly indicating that the connective tissue proliferation is still progressive. Only in those cases in which the kidney is markedly shrunken and granular does the peripheral portion of the connective tissue have a dense fibrous structure, and even in these there are usually scattered areas of cellular infiltration in which the cells consist partly of fibroblasts and partly of plasma cells.

The explanation of these phenomena is not clear. At first sight it might seem that the extreme degeneration and necrosis of the tubular epithelium in acute uranium poisoning must be responsible for the secondary proliferation of the connective tissue and for the marked glomerular changes. If we accept the teaching of Senator, namely, that destruction of any part of a tubule system will lead to secondary destruction of the remainder of that tubule system, we could explain the destruction of the glomeruli, and, perhaps also, the cystic dilatation of the glomerular capsules. But it is difficult to understand how simple destruction of the tubular epithelium could account for the peculiarly constant development of connective tissue in the inner zone of the cortex, or for the evident progression of the connective tissue proliferation which is found months after the insult which produced the primary necrosis. Again, if it is simply the epithelial necrosis which is responsible, one would expect to find equally severe secondary fibrosis in kidneys which have been poisoned with potassium bichromate, for in acute bichromate nephritis the lesions are practically indistinguishable from those which are produced by uranium nitrate. The experiments of Ophüls seem to show that this is not the case. He subjected forty guinea-pigs and fifty rabbits to repeated injections of potassium bichromate during periods varying from a few weeks to almost two years' duration. In his recent report[19] on the effects produced in guinea-pigs he says:

> The conclusion seems justified that it is impossible to produce severe lasting lesions in guinea-pigs with chromates, probably because in these animals the chromates are too toxic in a general way, and too slightly effective on the kidneys locally.

19. Ophüls, W.: Experimental Nephritis in Guinea-Pigs by Subcutaneous Injections of Chromates, Proc. Soc. Exper. Biol. and Med., 1911, ix, 13.

And in describing his experiments on rabbits he says:[20]

Rabbits are relatively more susceptible to the action of chromates than guinea-pigs. * * * The extreme lesions which may develop after a while as a result of crowding of large doses were described by me in 1908 (*Jour. Med. Research*, 1908, xviii, 49), but they do not necessarily follow even repeated administration of large doses. * * * Fifty animals were experimented upon. The dosage varied between two centigrams and two milligrams. Some of the animals were kept alive for a year or more. So far as epithelial lesions are concerned the experiences are similar to those in guinea-pigs, except that the epithelial lesions became more severe and seemed to continue longer after the last injection. Very marked interstitial lesions were observed at times, but they resemble those observed spontaneously so closely, and occurred so irregularly, sometimes soon, sometimes late after the administration of various doses and sometimes not at all, that any definite conclusion of their relation to the injections could not be arrived at.

It is true that the findings of Smith[2] differ considerably from those described by Ophüls. From a series of thirty-nine rabbits and seven guinea-pigs which survived for longer than three months after the initial injection of either potassium bichromate or uranium nitrate (the exact number of each is not stated), he concludes that "the chronic lesions caused by uranium nitrate and potassium bichromate were the same." and that "bichromate of potassium produces more fibrous tissue formation with macroscopic depressions on the surface of the kidney than uranium nitrate."

If these observations of Smith are based on a sufficiently large number of cases of bichromate nephritis, his results are of great interest in view of the large number of negative results described by Ophüls, and they would greatly weaken the argument that in uranium nephritis it is not alone the epithelial necrosis which is responsible for the secondary development of fibrous tissue. However, judging from the large number of experiments which have been conducted in our laboratory, there seems little doubt that in uranium nitrate nephritis there is some factor which is lacking in potassium bichromate nephritis, and which is responsible for the almost constant production of chronic kidney lesions in the former. Just what this factor may be is still in doubt. I am convinced that there is something more than mere coincidence in the fact that there is the occurrence of edema in acute uranium intoxication, and the development of chronic kidney lesions after prolonged uranium administration. If it be true that the formation of edema depends on a combination of lesions in the kidneys and in the peripheral blood-vessels, I believe there is no doubt that the development of the chronic kidney lesions is dependent on a similar combination of lesions. Indeed, I believe that no other condition than some damage to the vascular system can explain

20. Ophüls, W.: Experimental Nephritis in Rabbits by Subcutaneous Injections of Chromates. Proc. Soc. Exper. Biol. and Med., 1911, ix, 13.

the peculiarly characteristic distribution and the progressive type of interstitial connective tissue proliferation which we find in chronic uranium nephritis.

THE CARDIOVASCULAR CHANGES

The question of the interrelationship which exists between hypertrophy of the left ventricle of the heart and chronic interstitial nephritis is one which has received much attention. Various observers have approached the problem by simulating the local kidney conditions of chronic interstitial nephritis by the actual reduction of kidney substance by surgical methods. Bradford,[21] Passler and Heinecke,[22] Janeway,[23] Sampson and Pearce[24] and others have found that, following such reduction of kidney substance, there develops in certain cases a rise in blood-pressure and hypertrophy of the left ventricle of the heart. Rautenberg[25] also by mechanically damaging one kidney and subsequently removing the other kidney has succeeded in raising blood-pressure in rabbits to such an extent that aneurysms of the aorta are formed. But the number of observations of development of hypertrophy of the heart in cases of experimental chronic nephritis is very small. Charcot and Gombault[26] and Levaditi[27] report hypertrophy of the left ventricle of the heart in cases of experimental chronic nephritis produced by lead salts and vinylamin, respectively. Siegel[28] reports hypertrophy of the left ventricle of the heart in three dogs which had been subjected to uranium injections for from three to six weeks, and Smith[2] reports four cases of apparent heart hypertrophy in his series of uranium nitrate rabbits. However, the limited number of instances in which experimental chronic nephritis has been produced has of necessity limited the number of observations which could be made.

In a series of experiments such as those reported in this paper the difficulty of determining the presence of a true heart hypertrophy is very

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21. Bradford: The Results following Partial Nephrectomy, and the Influence of the Kidney on Metabolism. Jour. Physiol., 1899, xxiii, 415.

22. Passler and Heinecke: Versuche zur Pathologie des Morbus Brightii Verhandl. d. deutsch. pathol. Gesellsch., 1905, ix, 99.

23. Janeway: Note on Blood-Pressure Changes Following Reduction of the Renal Arterial Circulation. Proc. Soc. Exper. Biol. and Med., 1909, vi, 109.

24. Sampson and Pearce: A Study of Experimental Reduction of Kidney Tissue with Special Reference to the Changes in That Remaining. Jour. Exper. Med., 1908, x, 745.

25. Rautenberg: Erzeugung chronischer Neirenkrankungen mit folgender Blutrucksteigerung und Arteriosklerose. Deutsch. med. Wchnschr., 1910, xxxvi, 551.

26. Charcot and Gombault: Noté relative à l'Étude anatomique de la nephrité saturnine experimentale. Arch. de physiol., 1881, Series 2, viii, 126.

27. Levaditi: Experimentelle Untersuchungen über die Necrose der Nierenpapillæ. Arch. internat de pharmacodyn., 1901, viii, 5.

28. Siegel: Uber experimentelle Nephritis. Verhandl. d. Congresses Inn. Med., 1907, xxiv, 217.

great. In normal animals quite definite ratios of heart to body weight have been established, and in animals which have been subjected to experimentation but in which the weight at the time of death shows no marked variation from the weight during the time in which the animal has been under observation, similar heart to body ratios can be established and compared with those of normal animals. But in those animals which lose in weight during the course of the experiment, and especially in animals which decrease rapidly in weight during the terminal illness, such heart to body ratios are difficult to determine. One cannot tell exactly what proportional loss of weight occurs in the heart itself during the progressive loss in the whole body weight; in such cases one must rely largely on the macroscopic appearance of the heart. In well-marked cases of heart hypertrophy there is little difficulty in forming an opinion, but in doubtful cases, and especially in the smaller animals, one must be very guarded in making the decision that hypertrophy actually exists.

Conservative inspection of the hearts of the animals reported in this paper showed quite definitely that there was hypertrophy of the heart, especially of the left ventricle, in two guinea-pigs, four rabbits and nine dogs. In several other cases the heart gave one the impression of being hypertrophied, but the hypertrophy, if present, was too slight to justify a definite conclusion. In the rabbits and guinea-pigs no confirmatory heart to body ratios were established, but in the dogs such ratios were determined and were studied in comparison with those worked out in normal dogs by Stewart[29] and Joseph.[30] Table A shows clearly the result of this comparison.

In Stewart's series of twenty normal dogs he found that there was an average heart to body ratio of 0.00724, a ratio approximately the same as that obtained by Joseph, namely, 0.00750. But among Stewart's twenty animals there were three which showed a ratio of 0.00800 or over, and in his series of experiments he withheld a diagnosis of heart hypertrophy in all cases in which the ratio did not exceed the maximum ratio obtained in his normal dogs. It will be seen that some of my cases fall below the maximum normal ratio as estimated by Stewart, and may therefore be viewed with some suspicion. But when we consider that in all my cases the animals were confined to small quarters during practically the whole of their lifetime, and would presumably on that account show relatively smaller hearts than would active street dogs, the constantly high ratio is very striking. And it is of interest that my three normal controls, which were kept under similar conditions, all showed heart to body ratios which were well below the average as established by Stewart. It is also

29. Stewart: An Experimental Contribution to the Study of Heart Hypertrophy. Jour. Exper. Med., 1911, xiii, 187.

30. Joseph: The Ratio between the Heart Weight and the Body Weight in Animals. Jour. Exper. Med., 1908, x, 521.

of interest that in all but two of the cases included in the accompanying table, the weight at the time of death was as great or greater than at any time during the course of the experiment, due to the fact that these animals were intentionally or accidentally killed several weeks after the last injection. In two cases in which there was definite loss of body weight immediately preceding death, the ratio of the heart weight at the time of death to the maximum body weight during life was high, and I believe that one is justified in assuming that the actual heart to body ratio must lie somewhere above that ratio, but below the ratio of the heart weight at time of death to the body weight at time of death. Accordingly in the table these two ratios are entered as (a) and (b) and the actual heart to body ratio is assumed to lie somewhere between the two, in both cases at a point which is high as compared with the average normal ratio.

TABLE A.—(A) SHOWING THE RATIOS WHICH EXIST BETWEEN THE HEART WEIGHT AND THE BODY WEIGHT IN DOGS WITH CHRONIC URANIUM NEPHRITIS; (B) SHOWING SIMILAR RATIOS IN NORMAL DOGS

Protocol Number	Animal Number	Duration of Exp't, Weeks	Body Weight in Grams	Heart Weight in Grams	Ratio
2	3—1909	34	10,260	80	0.00779
3	4—1909	31	14,280	118	0.00802
5	6—1909	29	5,610	60	0.01069
6	1—1910	61	7,680	61.5	0.00800
7	2—1910	71	16,300	145	0.00888
8	3—1910	60	21,600	180	0.00818
9	5—1910	25	(a) 8,980	73	0.00822
..	.....	..	(b) 8,020	73	0.00922
17	24—1910	32	(a) 6,900	55	0.00790
..	.....	..	(b) 5,200	55	0.01041
			NORMAL CONTROL ANIMALS		
..	8—1909	..	8,640	60	0.00694
..	4—1910	..	8,400	60	0.00714
.	29—1910	..	4,680	34	0.00705
Stewart's Series—20 dogs					0.00721
Joseph's Series					0.00750

These weights and ratios are presented with the full realization that in some of them the proportional weight of the heart is somewhat below what may be considered the limit of normal variation. However, I believe that when we take into consideration the age of the animals which were used, the conditions under which they were kept, and the fact that in every case the heart showed macroscopic appearance of hypertrophy, one is justified in presenting them as evidence of proportional increase in the size of the heart.

The condition of the blood-vessels has already been described in considering the lesions which are found in the kidneys.

CONCLUSIONS

1. By the administration of uranium nitrate it is possible to produce a chronic diffuse nephritis in guinea-pigs, rabbits and dogs.

2. The histological picture of the lesions is characteristic, and is analogous to, but not identical with, that found in chronic diffuse nephritis in man.

3. The chronic lesions, even in the most severe cases, are not associated with demonstrable arterial lesions, although the mode of development suggests that there must be some functional vascular damage.

4. After prolonged administration in guinea-pigs a terminal attack of acute nephritis is frequently accompanied by more or less marked anasarca.

5. Associated with the more severe kidney lesions there is frequently a more or less marked hypertrophy of the left ventricle of the heart.

Sacramento and Webster Streets.

Reprinted from the Archives of Internal Medicine
May, 1912, Vol. 9, pp. 557-591

CHICAGO
AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE DEARBORN AVENUE
1912

No. 9

[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XV, No. 6, 1912.]

MALARIAL PIGMENT (HEMATIN) AS A FACTOR IN THE PRODUCTION OF THE MALARIAL PAROXYSM.*

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It is remarkable that, from the enormous amount of investigation that has centered about the malarial parasite and its relation to malarial fevers, there has come no clear exposition of the mode of production of the various phenomena of the malarial paroxysm. It is true that these phenomena have been ascribed to the presence in the circulation of some toxic substance, or substances, elaborated by the malarial parasite, and that the blood at the time of the segmentation of the parasite has been shown to possess toxic properties. Still, as far as I know, the nature of these toxic agents remains unknown, as no one has clearly demonstrated the presence of any definite substance, which, when introduced into the circulation, could reproduce the symptom complex of the malarial paroxysm. The observations embodied in this report are offered, therefore, with the hope of shedding some light upon the question.

In the course of some work on hematin metabolism, it was noted that a rabbit that had received an intravenous injection of alkaline hematin developed a very pronounced shaking chill, strikingly like that of malaria. As the author has attempted to show in a previous paper,[2] the pigment elaborated from the hemoglobin of the red blood corpuscle by the malarial parasite and liberated into the circulation of the host at the time of segmentation of the parasite is undoubtedly hematin. It at once appeared possible, therefore, that we might find in this substance one of the hypothetical toxins operative in malaria.

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, March 29, 1912.

[1] The first seven experiments of this investigation were done in the Pathological Laboratory of the University of Wisconsin, Madison, Wis.

[2] W. H. Brown, *Jour. Exper. Med.*, 1911, xiii, 290.

The investigation of this question now embraces a series of ninety observations upon the effect of the intravenous injection of alkaline hematin, eighteen rabbits having been used for test purposes.

TECHNIQUE.

Materials Used and Their Preparation.—The hematin used was derived from three sources, rabbit blood, dog blood, and ox blood, but in all cases it was prepared by the Schalfijew process. The solutions for injection were made in 0.85 per cent. salt solution containing 1.5 or 2 per cent. bicarbonate of sodium. The strength of the hematin employed has varied from 1.5 to 5 milligrams per cubic centimeter. The hematin was added to the sterile solvent and heated to 100° C. for five to ten minutes, and was then allowed to stand for twelve to twenty-four hours, when it was again heated and, while still hot, filtered into sterile flasks. The subsequent treatment of these solutions has varied somewhat. Solutions prepared thus, and again heated to 100° C. for five to ten minutes have apparently remained sterile. In a few instances, however, the filtered solution has been autoclaved to insure sterility.

It has been found extremely difficult to prepare hematin solutions of absolutely uniform character. With a given preparation of hematin and with the same technique in the preparation of the solutions, two distinct types of solution may be obtained: one, a perfectly clear, deeply colored solution that even under the microscope shows very few particles of pigment suspension, and on standing shows no precipitation in the flask; the other, a turbid solution that appears chocolate brown in thin layers and under the microscope shows myriads of pigmented particles and droplets floating in an only faintly colored fluid. Much of this pigment is precipitated on allowing the solution to stand. This latter "colloidal" type of solution or suspension, manifests all the optical properties of an alkaline hematin solution and the suspended pigment readily passes through Schleicher and Schüll filter paper No. 597 which has been used as the routine filter. With different preparations of hematin these variations in solubility are continually appearing. Reference is made to this feature of the hematin solution to indicate the

difficulty in maintaining absolutely uniform experimental conditions and accurate dosage.

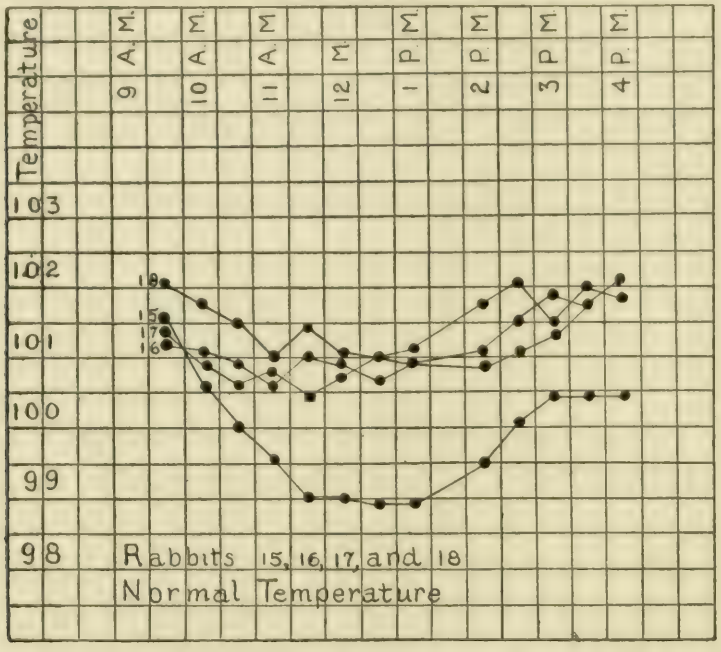
Experimental Procedure.—Briefly, the experiments have been conducted according to the following plan: The animals were accustomed to laboratory surroundings by being kept in cages from one to two days before any observations were made. The normal temperature curve of each animal was then established by rectal temperature, taken every half hour from 8 or 9 A. M. until 3 or 4 P. M., the time to be covered by subsequent experiments. As it seemed probable that the sodium bicarbonate salt solution used as the solvent for hematin might produce some toxic manifestations when injected intravenously, the action of this solution was determined in a number of instances, usually in such doses as were subsequently to be administered with the hematin. While these control tests were usually made prior to the injection of hematin, some such tests followed the administration of hematin. The animals were next given intravenous injections of alkaline hematin at 8 or 9 A. M., and the resulting phenomena were noted, the temperature being taken as previously, although fifteen minute observations were frequently made.

Dose of Hematin.—It is evident that the question of dosage in such a series of experiments is one of prime importance. To carry out the object of these experiments it is quite essential that the dose of hematin employed be somewhat comparable to that liberated into the human circulation at the time of segmentation of a generation of parasites. While there is no evidence to show that all the hemoglobin of the infected red corpuscles is decomposed to form hematin, if we may assume that such is the case, and, further, that a 1 to 5 per cent. infection of red blood corpuscles is not uncommon, we have sufficient data upon which to base a calculation of the approximate amount of hematin liberated into the human host with the segmentation of a given generation of parasites. Basing our calculations on the presence of 8.5 grams of hemoglobin per kilo of body weight, and 4.47 per cent. of hematin in hemoglobin,[3] we find that in a 1 per cent. infection of the red blood corpuscles approximately

[3] Olof Hammarsten, A Text Book of Physiological Chemistry, translated by Mandel, New York, 1901, p. 139. These figures are probably high.

3.7 milligrams of hematin per kilo of body weight would be liberated. In my experiments the dosage has varied from 1.3 milligrams to 28 milligrams per kilo of body weight, given in single or in divided doses, corresponding roughly to an infection of 0.3 to 7.5 per cent. of the red blood corpuscles.

Normal Temperature of the Rabbit.—In undertaking an investigation which must necessarily deal so largely with variations in the temperature of the experimental animal, it is imperative that the basis of comparison between normal and abnormal fluctuations of temperature be as free from objection as possible. Unfortunately, the temperature of different rabbits varies widely, even when kept under exactly the same conditions. In apparently normal animals I have found the individual extremes between 98° and 103° F. Likewise, the fluctuations of temperature in a given animal may be quite considerable, but usually follow a fairly definite course.



TEXT-FIG. 1. Normal temperature of four rabbits during the period of the experiments.

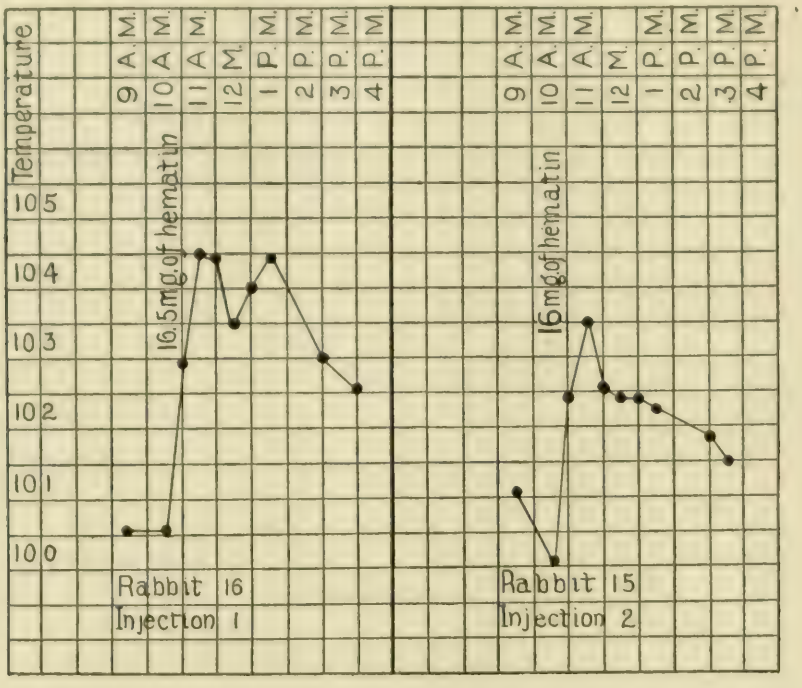
The course and fluctuations of temperature of the normal rabbit under experimental conditions, as well as the individual difference in temperatures, are shown in text-figure 1. This chart shows the normal temperatures recorded for rabbits 15, 16, 17, and 18. The

first three animals were from the same litter, about three quarters grown, and weighed 1,600 to 1,700 grams. Number 18 was full grown and weighed 1,840 grams. All the records were taken at the same time and all conditions were as nearly alike as possible. While three of these curves coincide closely, the fourth shows an extremely low and irregular curve of temperature. It should be noted that the temperature in all instances has a downward trend during the morning hours, and does not show an upward tendency until about noon, when there is a gradual rise, which ultimately reaches as high as the temperature at the first observation or even higher. This temperature curve has been fairly constant in my entire series of experiments.

Effect of Hematin upon Temperature.—If, for purposes of comparison, we adopt the classical division of the malarial paroxysm into a cold stage, a hot stage, and a stage of sweating, with the concomitant symptoms belonging to each, certain of these manifestations are capable of accurate measurements in an experimental animal, while others may be determined with a fair degree of accuracy by close observations, and still others are wholly indeterminable. Of prime importance among these phenomena of the malarial paroxysm is the question of fever.

In estimating the temperature effects, in all instances at least three facts are to be taken into consideration; the nature of the effect, the degree of the effect, and the duration of the effect. While it has been possible to assemble much of the data concerning the effects of hematin upon the temperature in an appended table which shows the abbreviated protocols of the entire series of experiments, it must be fully appreciated that such tabulations of statistics are wholly inadequate to present many features of the experiments that are equally as important as those thus presented, and attention will be especially directed to such features. Further, as can be seen from these tables, it has been the object of the author to study effects in individual animals with a variety of doses, as occasion suggested, rather than to mould all the experiments to a single type or plan, for it became evident early in this investigation that individual peculiarities of the animals played a prominent rôle in the results obtained.

Without exception, every dose of hematin administered has elicited a definite temperature response. With but three exceptions, this response has been characterized by a sharp rise in temperature, reaching the fastigium in about an hour and a quarter. The further course was somewhat variable, although in most cases with a dose of 15 milligrams, or less, per kilo, there was a rather sharp fall of temperature for thirty minutes to one hour, followed by a secondary rise of variable extent and duration or a very gradual decline requir-



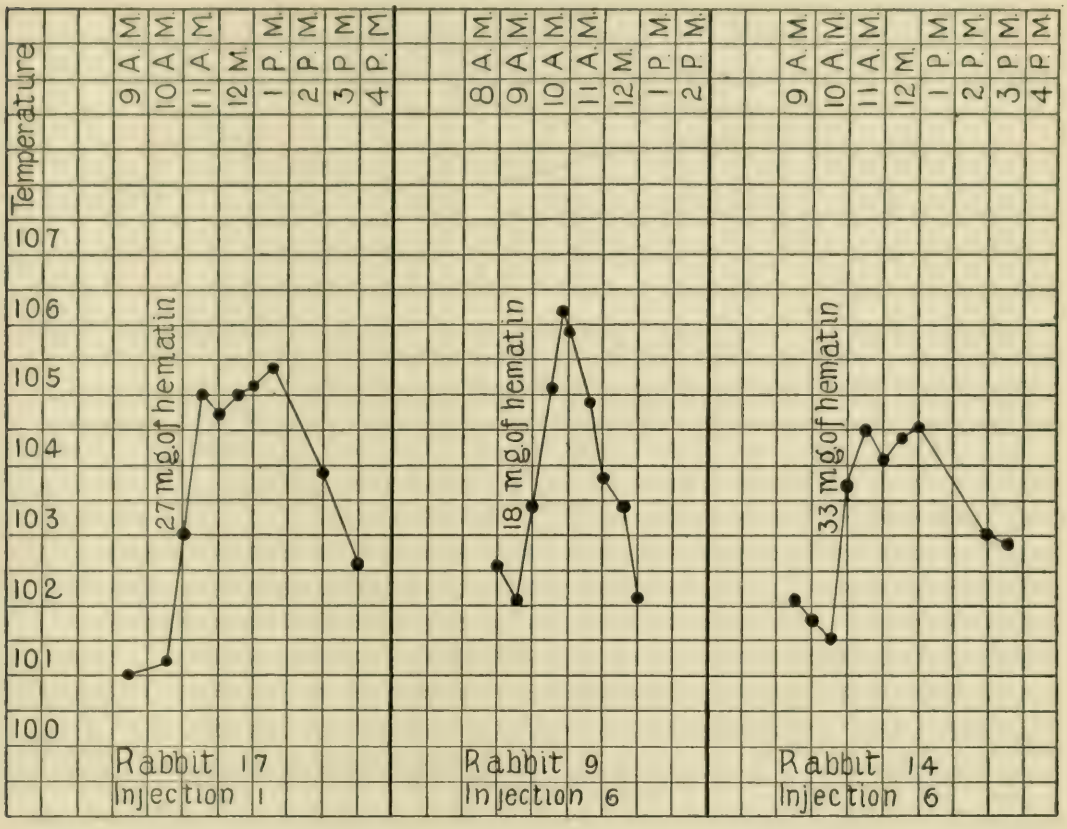
TEXT-FIG. 2. The usual types of temperature curve following injections of hematin.

ing several hours to reach normal again. Two such temperature curves are shown in text-figure 2.

These curves are subject to innumerable variations depending upon the dose, the stage of the experiment, and upon the individual peculiarities of animals. Some of the more important variations are an accentuation and prolongation of the secondary rise, usually shown with initial and large doses, or a defervescence that is almost as sharp as the rise in temperature. A third variation, which includes the three exceptions previously noted, is met with in instances

of marked intoxication and is characterized by an initial drop in temperature with a subsequent rise. All three of these modifications are illustrated in text-figure 3.

The extent of the temperature elevation is, within certain limits, proportional to the amount of hematin injected. The temperature effect, being very slight with small doses, increases with the dose,



TEXT-FIG. 3. Variations in the temperature curve following large doses of hematin or repeated injections of hematin.

until we begin to obtain signs of an over-intoxication when the elevation may be much less than with smaller doses, the optimum dose usually being between 10 and 15 milligrams per kilo of body weight. The elevation of temperature obtained with such optimum doses is from 3° to 3.5° F., and it is exceptional that a greater rise is reached. Occasionally, however, the temperature may rise 4° F. or even higher in highly susceptible animals. The greatest elevation recorded in my series of experiments was 4.9° F. in animal 9, with a dose of 18 milligrams of hematin per kilo.

In well-marked reactions, the temperature usually returns to within the normal range in the course of three to five hours. With large or initial injections of hematin, the period of elevated temperature is more prolonged than under other circumstances and seldom reaches normal in less than four hours, occasionally requiring as much as six hours. Exceptionally, the return to normal may be rapid (text-figure 3).

The method of administration also plays an important part in the results. A given dose of hematin injected in two or three fractional doses at intervals of fifteen to thirty minutes produces a much more marked elevation of temperature than when given at a single injection, an effect that is well shown in rabbit 13. This is particularly true of the smaller, or optimum doses, while with larger doses the increased potency may be manifested by a slowing of the rise of temperature, a cessation of the rise, or even a fall upon the injection of the second fraction, as illustrated in rabbit 12.

Neither the source of the hematin nor the type of solution seems to play an important part in the results that I have obtained. That is, there has been but slight difference between the action of rabbit, dog, or ox hematin, or between the action of the perfectly clear hematin solution and that containing much finely divided hematin in suspension.

However, a few tests seem to indicate that solutions of hematin gradually lose their pyrogenic properties with age or when subjected to high and prolonged temperature. This apparent decrease may be seen by comparing the results obtained in rabbit 7, injection 3, and rabbits 8, 10, and 11. Further, it can readily be imagined that all animals will not be found equally susceptible to the toxic action of hematin. A few will exhibit a marked sensitiveness and a few will be found extremely resistant, the optimum dose in the one producing but slight effect in the other. This variation in susceptibility was strikingly illustrated by animals 13 and 14 which were under observation at the same time. Rabbit 13 was a typically resistant animal, and rabbit 14 was highly sensitive.

Effect of Sodium Bicarbonate Salt Solution upon the Temperature.—Animals injected with the bicarbonate salt solution alone, for purposes of control, almost invariably showed an elevation of tem-

perature in proportion to the size of the dose, and about one third to one half the elevation produced with an equal amount of hematin solution, depending somewhat upon the concentration of the hematin in the solution. With small doses of the control medium, the fluctuations of temperature were usually within what might be termed the normal range and were such that it is difficult to say whether they are more than incidental to the process of injection. Large doses may produce a rise in temperature corresponding approximately to the over-intoxicating effect of large doses of hematin. In such instances, however, other features of the clinical picture will distinguish sharply between the two cases. In all instances, therefore, there were distinct differences between the action of the sodium bicarbonate salt solution and the action of the hematin solution, such that there can be no question as to the part played by the hematin in these experiments.

Other Phenomena of the Hematin Paroxysm.—Apart from the elevation of temperature in the experimental animal, the paroxysm of hematin intoxication presents other features which are of equal importance and show a strong resemblance to corresponding phenomena of the malarial paroxysm. For the first fifteen to twenty minutes following the injection of hematin, the rabbit usually manifests a slight degree of restlessness, then crouches in a corner of the cage. In the second stage of the paroxysm the vessels of the ears contract giving to the shaved ears a pale and cyanotic hue, while at the same time the ears become decidedly cold. In pronounced cases the surface temperature (temperature of the ears) may be more than 30° F. below the rectal temperature. The lowest temperature recorded in this series of experiments was 63.5° F. with a room temperature of 62.5° F. and a rectal temperature of 105° F. During this stage the animal's ears usually lie on its back, and the hair tends to become erect, presenting the picture of an animal that is cold. Meanwhile, the rabbit shows convulsive tremors or shivering, but rarely any continued or pronounced shaking. This stage of chill lasts from forty-five minutes to one hour, and is terminated rather abruptly by a dilation of the superficial vessels, the ears rapidly becoming flushed and hot. The animal now moves about the cage or stretches out and remains quiet. Further than this, the

third or hot stage of the paroxysm possesses no especial symptoms and its limit can be fixed only by the course of the temperature, which may remain well above normal for several hours, or sink to normal within an hour. During the third stage and the latter part of the second stage of the paroxysm the animal shows a pronounced thirst which is undoubtedly referable to the febrile condition.

The most striking and at the same time the most constant of all these symptoms are the contraction and dilatation of the superficial vessels associated with the corresponding lowering and elevation of the surface temperature.

The contrast between the symptoms of the animal injected with hematin and those of control animals is quite as sharp as in the instance of the temperatures. With doses of hematin sufficiently large to produce pronounced symptoms of the type described, corresponding doses of sodium bicarbonate salt solution produce practically no recognizable effect. There may be a suggestive or very transient change in the surface vessels and the temperature, but nothing that is definite or constant. When, however, larger doses, *e. g.*, ten cubic centimeters per kilo, are given, phenomena simulating the picture of hematin intoxication may be elicited, but the changes are not so definite or constant.

If now we correlate these symptoms with the temperature curve, we find that the elevation of temperature during the first stage is slight, and that the second stage of the paroxysm corresponds closely with the period of rising temperature, the initial drop coinciding sharply with the vascular dilatation and flushing of the ears and the elevation of the surface temperature. The third or hot stage, as previously noted, corresponds to the duration of the temperature above normal.

As in the case of the temperature, all other phenomena of hematin intoxication seem to be exaggerated when a given dose of hematin is divided into several fractional doses, the cycle of phenomena following closely consequent changes in the temperature curve.

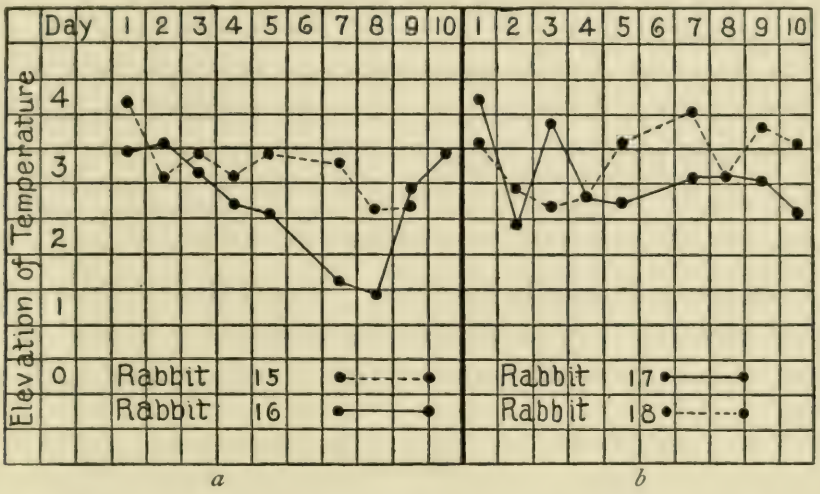
It must be pointed out, however, that the prominence of these paroxysmal phenomena and the degree of elevation of the temperature are by no means always parallel. The toxic paroxysmal phenomena may be present to a high degree in an animal that shows

only a slight elevation of temperature, and in most instances such a condition is to be regarded as evidence of over-intoxication.

Acquired Resistance.—Early in the course of these experiments it became evident that repeated injections of a given dose of hematin in the same animal did not give uniform results. The results, however, were of such a nature as to suggest that the animal acquired a certain degree of tolerance which, in turn, might be broken when the intoxication was pushed sufficiently. To determine this point the following experiment was carried out.

Experiment.—Four rabbits, weighing respectively 1,600, 1,650, 1,790, and 1,840 grams, were injected on ten successive days with a solution of ox hematin containing 5 mg. of hematin to 1 c.c. The first two animals received 10 mg. of hematin per kilo of body weight, and the other two received 15 mg. per kilo. Rectal temperatures were recorded every half hour.

The results are shown in text-figure 4. In *a* is shown the differences of elevation of temperature on successive days of the two



TEXT-FIG. 4. Variations in the elevation of the temperature with repeated injections of a given dose of hematin.

animals receiving ten milligrams per kilo, and in *b* those receiving fifteen milligrams per kilo.

While the curve of temperature reaction in each case is extremely irregular, it is in general characterized by a tendency towards a decrease, which in the instance of animals 16 and 17 persists throughout the experiment. With animals 15 and 18, however, a second phase of increased reaction is developed. These animals also

exhibited the most marked symptomatic effects throughout the experiment. If the temperature curve alone be considered, it is certain that the tendency is toward a decreasing response to successive injections of hematin and this I have found to be true in other experiments. If we take into consideration other evidence of intoxication, however, as in animals 15 and 18, this decrease seems referable not so much to a tolerance as to over-intoxication. Again, as these symptoms of intoxication decrease and the fluctuations of temperature increase correspondingly, there may be developed a certain degree of tolerance. On the other hand, as shown in animals 16 and 17, there may be an increasing resistance to the hematin from the start as the toxic symptoms as well as the temperature decrease proportionally.

This subject of acquired tolerance has been taken up largely to emphasize the importance to be attached to results obtained from properly adjusted initial doses of hematin but also to explain the apparent discrepancies in the results from any series of hematin injections. It is the initial injection, with but few exceptions, that gives the maximum temperature reaction obtainable with a given dose of hematin until the series of injections has been extended to such a degree as to permit of the acquirement of a tolerance in highly susceptible animals or to cause a break in the early acquired tolerance of more resistant animals. When such conditions supervene, the temperature reaction may again increase and show an even greater response than with the initial injection (table I, animals 9 and 18).

SUMMARY.

The paroxysm of hematin intoxication in the rabbit undoubtedly presents many features of striking similarity to the paroxysm of human malaria; still one must hesitate to apply such results unreservedly in an attempt to identify the causative agent of the malarial paroxysm. When, in addition to the character of the paroxysm, we consider the sequence of events in the two instances, the analogy becomes so close that it seems impossible to regard the matter as a mere coincidence. The injection of hematin, especially in fractional doses, is in a measure comparable to the liberation of hematin into the human circulation by the malarial parasite. In these experi-

TABLE I.

Summary of Experimental Data.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 1. Weight, 2.49.	Jan. 27, 1911	5.0			102.3	1.1		2	102.6	Rabbit hematin in all injections.
	Jan. 30, 1911		5.0	3.0	102.5		2.5	3	105.0	Highest temperature on secondary rise.
	Jan. 31, 1911		10.0	6.0	101.8		2.2	1½	102.8	
	Feb. 1, 1911		5.0	3.0	103.0		1.4	1½	103.0	
	Feb. 2, 1911		20.0	12.0	103.0		0.0		96.4	In two doses, 5 minutes apart. Died 3½ hours after injection.
No. 2. Weight, 2.78.	Feb. 1, 1911	10.0			102.8	1.8		2½	104.3	
	Feb. 2, 1911	10.0			102.2	1.8		1½	103.0	Rabbit hematin.
	Feb. 3, 1911	23.0			102.1	2.5		1	102.8	
	Feb. 7, 1911		5.0	2.6	102.9		3.1	1¼	106.0	Initial rise to 106° with fall to 105.3° F. and second rise.
	Feb. 8, 1911		2.5	1.3	102.6		1.2	1	103.0	
	Feb. 9, 1911		7.5	3.9	102.5		2.6	1¼	104.0	
	Feb. 11, 1911		7.5	3.9	102.4		3.0	1¼	103.9	
	Feb. 15, 1911		15.0	7.8	102.3		4.0	1¼	106.1	
	Feb. 16, 1911		20.0	10.4	102.8		3.3	1¼	104.9	
No. 3. Weight, 2.9.	Feb. 8, 1911		2.5		102.7					2.5 c.c. thick suspension of hematin in 0.85% salt solution. Died instantly.
No. 4. Weight, 2.65.	Feb. 8, 1911	10.0			102.6	1.4		1½	102.8	
	Feb. 9, 1911		7.5	4.2	102.2		2.6	1¼	104.6	Rabbit hematin.
	Feb. 11, 1911		7.5	4.2	102.3		2.8	1¼	103.8	
	Feb. 15, 1911		10.0	5.7	102.4		2.5	1¼	104.8	

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours After Injection.	Remarks.
No. 4. Weight, 2.65.	Feb. 16, 1911		15.0	8.4	101.6		2.1	3	103.7	Temperature dropped to 100.7° F., then rose very slowly.
No. 5.	May 1, 1911	7.0			101.8	1.3		1½	102.2	
Weight,	May 2, 1911		7.0	5.0	102.0		3.6	1½	105.0	Dog hematin.
2.85.	May 3, 1911		7.0	5.0	102.3		3.2	1¼	104.7	
No. 6.	May 1, 1911	8.0			102.4	1.6		1½	103.0	
Weight,	May 2, 1911	10.0			101.8	1.2		1½	102.2	Other manifestations very slight.
2.57.	May 3, 1911	15.0			102.0	1.7		1¼	103.3	
	May 4, 1911	20.0			102.1	2.1		1½	103.5	
No. 7.	May 1, 1911	8.0			101.6	1.5		2	102.0	
Weight,	May 2, 1911		8.0	4.7	101.2		3.7	1¼	104.0	Rabbit hematin.
2.53.	May 3, 1911		8.0	6.3	101.3		3.1	1¼	103.6	Dog hematin.
No. 8.	Oct. 18, 1911	6.0			102.1	1.4		1½	102.8	Hematin solution 5 months old, same as that used in in No. 7, injection 3.
Weight,	Oct. 20, 1911		6.0	10.0	102.3		1.8	1½	103.0	
1.85.										
No. 9.	Oct. 20, 1911	12.0			102.5	2.0		1½	102.7	
Weight,	Oct. 23, 1911		12.0	12.0	102.3		3.3	1	103.4	Ox hematin in all injections.
2.05.	Oct. 25, 1911		12.0	12.0	102.5		2.7	1¼	102.7	
	Oct. 26, 1911		12.0	12.0	103.6		2.0	¾	103.0	An afternoon experiment.
	Oct. 27, 1911		12.0	12.0	102.1		3.7	1	103.1	Temperature back to 103.6°
	Nov. 1, 1911		12.0	12.0	102.1		4.1	1¼	103.4	in 1½ hours.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 9. Weight, 2.05.	Nov. 3, 1911		12.0	12.0	101.8		4.3	1½	——	
	Nov. 6, 1911		18.0	18.0	101.5		4.9	1	103.5	Given in two doses 5 minutes apart.
No. 10. Weight, 1.85.	Oct. 25, 1911		12.0	20.0	102.6		0.8		103.0	Hematin solution 5½ months old, same as No. 7, injection 3, and No. 8, injection 2.
	Oct. 27, 1911		12.0	20.0	102.2		1.5		102.8	
No. 11. Weight, 2.0.	Oct. 27, 1911		5.0	7.5	102.6		0.6	1	102.5	Hematin solution, same as No. 10.
	Nov. 3, 1911	12.0			101.6	1.3		1½	101.6	
	Nov. 6, 1911	18.0			101.0	2.4		2	102.2	Ox hematin in all other injections.
	Nov. 8, 1911		6.0	6.0	101.4		2.6	1	102.8	
	Nov. 10, 1911		12.0	12.0	100.4		3.0	1¾	102.8	
	Nov. 13, 1911		18.0	18.0	100.3		3.9	3	104.2	
	Nov. 15, 1911		10.0	20.0	100.5		4.5	1½	103.0	
	Nov. 17, 1911		8.0	16.0	100.8		3.2	1¼	103.2	
No. 12. Weight, 2.0.	Nov. 10, 1911		15.0	15.0	101.2		3.2	1½	103.9	Ox hematin.
	Nov. 13, 1911		18.0	18.0	101.3		4.0	1½	105.0	
	Nov. 15, 1911		10.0	20.0	101.0		3.3	1	102.9	
	Nov. 17, 1911		8.0	16.0	101.7		3.1	1¾	104.0	
	Nov. 20, 1911		10.0	20.0	102.5		1.9	1½	103.3	In two doses 30 minutes apart. Initial rise checked by second dose.
	Nov. 22, 1911		5.0	10.0	101.4		3.4	1¼	103.4	Apparently about the optimum dose.
	Nov. 24, 1911		4.0	8.0	101.5		4.2	1¼	104.0	
	Nov. 27, 1911		8.0	16.0	101.4		3.0	1½	103.1	

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 13. Weight, 2.15.	Nov. 22, 1911	5.0			102.2	0.4		1	101.8	Ox hematin.
	Nov. 24, 1911		4.3	10.0	102.0		1.5	1	102.2	Single dose.
	Nov. 27, 1911		4.3	10.0	102.2		3.3	1¼	102.5	In 2 doses 30 minutes apart.
	Nov. 29, 1911	4.3			102.2	1.4		1¾	102.7	In 2 doses 30 minutes apart.
	Dec. 4, 1911	4.3			101.6	1.4		2½	102.8	In 2 doses 30 minutes apart.
	Dec. 6, 1911		4.3	10.0	101.8		3.0	1¼	103.6	In 2 doses 30 minutes apart.
	Dec. 8, 1911		2.1	5.0	101.7		2.1	1½	102.6	In 2 doses 30 minutes apart.
	Dec. 11, 1911		10.0	23.0	101.5		2.9	1¼	103.8	In 2 doses 30 minutes apart.
	Dec. 13, 1911		10.0	23.0	102.1		2.6	1	103.0	Single dose.
	Dec. 15, 1911		12.0	28.0	101.5		3.5	1	103.5	Single dose.
	Dec. 18, 1911		12.0	28.0	101.4		4.3	1¼	104.4	Given in 2 doses 30 minutes apart.
No. 14. Weight, 2.25.	Dec. 6, 1911	4.5			100.7	1.9		2¼	102.2	Ox hematin. Given in 2 doses 30 minutes apart.
	Dec. 8, 1911	2.2			100.4	1.6		2	——	Given in 2 doses 30 minutes apart.
	Dec. 11, 1911		2.2	5.0	101.0		3.2	1¾	103.4	Given in 2 doses 30 minutes apart.
	Dec. 13, 1911		3.3	7.5	101.4		3.4	1¾	103.6	Given in 3 doses 30 minutes apart.
	Dec. 15, 1911		4.5	10.0	101.5		2.6	1½	103.0	Given in 2 doses 30 minutes apart. Over-intoxication.
	Dec. 18, 1911		6.6	15.0	101.7		2.8	1½	104.5	Single dose. Over-intoxication.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours After Injection.	Remarks.
No. 15.	Jan. 12, 1912	3.2			100.6	1.5		1½	100.6	
Weight,	Jan. 16, 1912		3.2	10.0	99.8		3.2	1½	102.5	Ox hematin.
1.6.	Jan. 17, 1912		3.2	10.0	100.1		3.4	1½	102.3	
	Jan. 18, 1912		3.2	10.0	101.0		3.0	1	101.8	
	Jan. 19, 1912		3.2	10.0	101.2		2.5	1	102.6	
	Jan. 20, 1912		3.2	10.0	101.0		2.4	1½	101.9	
	Jan. 21, 1912									Another injection here without a record of temperature.
	Jan. 22, 1912		3.2	10.0	101.6		1.5	1½	102.0	
	Jan. 23, 1912		3.2	10.0	101.5		1.2	2½	102.7	Initial fall in temperature.
	Jan. 24, 1912		3.2	10.0	101.5		2.7	1	103.3	
	Jan. 25, 1912		3.2	10.0	101.8		3.2	1	104.0	
No. 16.	Jan. 12, 1912	3.3			101.1	2.7		1½	102.3	
Weight,	Jan. 16, 1912		3.3	10.0	100.6		3.9	1	104.4	Ox hematin.
1.65.	Jan. 17, 1912		3.3	10.0	101.8		2.8	1	102.9	
	Jan. 18, 1912		3.3	10.0	102.1		3.1	1	101.8	
	Jan. 19, 1912		3.3	10.0	102.0		2.9	1½	103.3	
	Jan. 20, 1912		3.3	10.0	102.5		3.2	1½	103.0	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		3.3	10.0	102.0		3.0	1½	103.1	
	Jan. 23, 1912		3.3	10.0	102.1		2.6	1½	103.0	
	Jan. 24, 1912		3.3	10.0	102.3		2.4	1	——	Leg broken by accident. Discontinued.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 17.	Jan. 12, 1912	5.4			101.2	2.5		1½	103.2	Ox hematin.
Weight, 1.79.	Jan. 16, 1912		5.4	15.0	101.3		4.0	3	105.3	Temperature reached 105° F. in 1 hour.
	Jan. 17, 1912		5.4	15.0	101.8		2.2	1	102.7	
	Jan. 18, 1912		5.4	15.0	101.5		3.6	1	102.6	
	Jan. 19, 1912		5.4	15.0	102.0		2.6	1½	103.3	
	Jan. 20, 1912		5.4	15.0	102.0		2.6	1½	103.3	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		5.4	15.0	102.2		2.8	1½	103.2	
	Jan. 23, 1912		5.4	15.0	102.1		2.9	1½	103.3	
	Jan. 24, 1912		5.4	15.0	102.6		2.8	1	103.3	
	Jan. 25, 1912		5.4	15.0	102.6		2.6	1	103.1	
No. 18.	Jan. 12, 1912	5.5			101.5	2.6		2	103.2	
Weight, 1.84.	Jan. 16, 1912		5.5	15.0	101.6		3.4	3	105.0	Ox hematin.
	Jan. 17, 1912		5.5	15.0	102.0		2.8	1½	103.5	
	Jan. 18, 1912		5.5	15.0	101.9		2.5	1	103.6	
	Jan. 19, 1912		5.5	15.0	102.3		2.6	1½	103.8	
	Jan. 20, 1912		5.5	15.0	101.5		3.4	1½	103.7	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		5.5	15.0	101.9		3.8	1	103.4	
	Jan. 23, 1912		5.5	15.0	102.2		2.8	1½	104.7	
	Jan. 24, 1912		5.5	15.0	102.5		3.6	1	103.7	
	Jan. 25, 1912		5.5	15.0	102.7		3.3	1	104.6	

ments, both solution and finely divided suspensions of hematin have been found equally effective in eliciting the phenomena of the paroxysm, and while it seems possible that a portion of the malarial pigment might be dissolved in the alkaline human serum, such an assumption is probably not essential.

It might be objected that the toxic action of foreign hematin thus injected into the circulation would probably be greater than that of hematin derived from an animal's own blood, but as far as I have been able to determine, this objection does not seem valid, as rabbit hematin, dog hematin, and ox hematin produce in the rabbit effects that are alike in both character and degree.

The dose of hematin remains as the one factor to which it is possible to attach some degree of uncertainty, but even here the author feels that the range of experimental conditions has been kept within the bounds of legitimate analogy with conditions existing in the human subject of malarial infection.

Finally, the most conservative estimate of the value of such experiments points strongly to the fact that we have at least a potentially toxic substance in the pigment hematin as liberated by the malarial parasite into the circulation of the human host.

There is also abundant evidence to show that the action of hematin is not confined to the paroxysmal phenomena of malaria, but that other features of the disease may find their explanation in the action of this pigment. For the present, however, it seems advisable to confine the discussion to this one phase of the question.

CONCLUSIONS.

1. Alkaline hematin in doses commensurate with the amounts of hematin liberated in the human circulation by the segmentation of the malarial parasite, produces, when injected intravenously into the rabbit, a paroxysm which is characterized by a short prodromal stage, a stage of chill and rising temperature, and a hot stage. In their details the phases of this paroxysm are practically identical with the corresponding ones in the paroxysm of human malaria.

2. The phenomena in human beings infected with malaria are, at least in part, directly referable to the toxic action of this malarial pigment.

A BIOCHEMICAL STUDY OF THE PHENOMENA KNOWN AS COMPLEMENT-SPLITTING

By JACOB BRONFENBRENNER and HIDEYO NOGUCHI

[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XV, No. 6, 1912.]

A BIOCHEMICAL STUDY OF THE PHENOMENA KNOWN AS COMPLEMENT-SPLITTING.

FIRST PAPER: SPLITTING OF THE COMPLEMENT ASSOCIATED WITH GLOBULIN PRECIPITATION.*

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INTRODUCTION.

During the last few years much attention has been directed toward a phenomenon known as the splitting of complement. The conception is that complement, when treated according to certain procedures, can be decomposed into two definite portions each of which is by itself inactive but can act in combination with the other as complement. The two components of the complement differ in their behavior in hemolytic processes; one has the property of being bound by the sensitized erythrocytes, while the other possesses a lytic action upon sensitized cells that have been acted upon by the first component. Thus, according to certain investigators, complement is composed of an intermediary portion, called the mid-piece, and a lytic portion, called the end-piece. The terms "mid" and "end" are employed because the conception is that the mid-piece stands between the sensitized cells and the lytic portion of the complement, while the lytic portion when viewed graphically, becomes the end-piece.

In view of the high lability of complement it is natural to ask if the phenomenon of splitting brought about by certain procedures may not be due to some modifications of the complement, such as a reversible inactivation through changes in the reaction of the medium in which the complement is present. It appeared to us of interest to approach this question from a biochemical standpoint.

In our first paper we deal with the splitting phenomena which are

* Received for publication, April 1, 1912.

associated with the precipitation of the globulin fraction, and in the second with those which occur without visible change in the physical conditions of the serum proteids.

HISTORICAL.

In fresh blood serum Landois found a hemolytic action upon alien corpuscles, and Fodor, Nuttall, and others, a bactericidal property, but none of the early investigators made exhaustive studies upon the nature of the active principles.

It was Hans Buchner who first pursued the study in a systematic manner and threw much light on this interesting phenomenon. Buchner (1890) established the fact that the active principle causing bacteriolysis or hemolysis is very labile and can be made inactive by a temperature of 55° C., by dialysis, or by dilution with distilled water. The active principle was designated by him alexin.

Bordet (1899) subsequently found that the alexin of Buchner is composed of two distinct principles, one a sensitizing substance and the other a thermolabile alexin. Somewhat later Ehrlich and Morgenroth (1899) made a similar observation, but by Ehrlich the sensitizing portion was called the amboceptor, and the alexin the complement. In this study Ehrlich and Morgenroth mention that the complement becomes inactive when mixed with water. Markl (1902) observed that sodium chlorid in concentration above 3 per cent. suppresses the activity of the complement.

While the disappearance of the complement activity after heating the serum to 55° C. was recognized by all as due to the destruction of the active principle at this temperature, no one was able to explain why the complement should lose its action in distilled water. This question was difficult to approach inasmuch as in a hypotonic salt solution a hemolytic experiment could not be carried out. Ferrata (1907), however, utilized the well known fact (noted by Hamburger, Madsen, and Arrhenius), that the corpuscles can be kept intact when suspended in an isotonic solution of sugar, and he studied the action of the salt-free complement (dialysed) in a salt-free isotonic medium, and found the complement to be inactive in this medium. Ferrata found also that during dialysis the serum separates into two portions, one the serum globulin (precipitate) and the other the albumin fraction (in solution). When tested separately in an isotonic salt solution neither of them caused hemolysis, while when united the action of complement was restored. He affirms that the component of complement remaining in the albumin portion is inactivated at 55° C.

The finding of Ferrata was confirmed by Brand, who showed further that the component carried down with the globulin fraction attaches itself to the sensitized corpuscles and that the component in the albumin fraction will afterwards act upon the cells so altered, He introduced, therefore, the terms mid-piece (Mittelstück) for the former and end-piece (Endstück) for the latter.

According to Brand the mid-piece of complement remains active when kept as precipitate (globulin) in distilled water, but loses its property on standing in salt solution. Brand and Tsurusaki (1908) found the mid-piece as well as the end-piece inactivated at 55° C.

Hecker (1907) and Sachs and Bolkowska state that the binding of the mid-

piece takes place at 0° C. as well as at higher temperatures, while that of the end-piece occurs only at higher temperatures. Thus they claim that the selective absorption of the natural hemolysin by means of corpuscles at 0° C. effects not only the fixation of the amboceptor, but also the mid-piece of complement.

Sachs and Teruuchi (1907) observed that the complement loses its activity in distilled water or isotonic sugar solution when the degree of dilution with distilled water is about 1 : 10, but not in much higher dilution. They were inclined to believe that the inactivation of the complement was caused by a certain ferment of the serum capable of exerting an injurious action only in a salt-free medium. They state that the inactivation of complement in a salt-free medium does not take place at a low temperature or in the presence of an excess of amboceptor. Complement a few days old does not undergo similar alteration (inactivation with water, etc.).

Tsurusaki found that the so-called splitting of complement is quite inconstant and seems to depend upon the quality of the dialysing membranes, upon the variations in different individuals of the same species of animal, and upon the temperature. For instance, the unaltered complement of rabbit was found in the supernatant fluid when dialysed at 0° C.

Sachs, and Sachs and Altmann (1908) then introduced a new method of splitting the complement. They used a weak solution of hydrochloric acid by which a certain portion of serum is precipitated (globulin). The precipitate corresponds with the mid-piece, and the supernatant fluid with the end-piece obtained by dialysis.

Later Michaelis and Skwirsky found that when corpuscles, amboceptor, and complement were put together in a medium containing a certain quantity of NaH_2PO_4 and Na_2HPO_4 in proportions resulting in isotonicity, there was no hemolysis. But on further analysis, they found that in this mixture the corpuscles had fixed not only the amboceptor, but also the mid-piece of complement. The corpuscles so modified were called persensitized, because they readily underwent hemolysis when the neutralized supernatant fluid (containing the end-piece) was added. In other words, persensitization can take place in a medium containing an excess of NaH_2PO_4 which suppresses the action of the end-piece, while the activity of the end-piece can be restored by neutralizing the excess of the acid phosphate with Na_2HPO_4.

Liefmann and Cohn introduced carbon dioxid for splitting the complement, whereupon the mid-piece is precipitated and the end-piece remains in solution. This phenomenon occurs only in a serum diluted with a sufficient quantity of water. They studied the influence of certain acids and alkalis and concluded that carbon dioxid exerts a far more inactivating effect than the acids or alkalis studied by them. Cholesterin inactivates the mid-piece in a salt-free medium. From the fact that the persensitization of corpuscles is affected with a minute amount of the mid-piece, they were inclined to think that this component acted like a ferment, an assumption held also by Rusznyák.

Jacoby and Schutze state that the complement inactivated by shaking and also the precipitate that is formed may be reactivated either by the mid-piece or the end-piece. On the other hand, the supernatant fluid of the shaken complement becomes active only with the end-piece.

Complement is known to be carried down by certain colloidal substances

through an adsorption (Landsteiner). Skwirsky confirmed this and points out a very interesting difference existing between the colloidal adsorption and the specific complement fixation as it occurs in the Bordet-Gengou or the Wassermann reaction. According to this author, later confirmed by Amako, in the former instance the end-piece as well as the mid-piece is carried down from the solution, while in the complement fixation the mid-piece alone is absorbed, leaving the end-piece free in the supernatant fluid.

Landsteiner, Liefmann and Cohn, and Fränkel state that the mid-piece prepared by the hydrochloric acid or carbon dioxid method from sera of different animals can persensitize the corpuscles for the end-piece of guinea pig serum, but not *vice versa*. This phenomenon is ascribed by them to the presence of natural amboceptor in the fraction containing the mid-piece. Fränkel points out certain differences in the behaviors of mid-pieces derived from the sera of different animals when these are acted upon by cholesterin, or sodium chlorid, or inactivated by the temperature of 57° C.

Marks observed that the reactivation of the end-piece by the mid-piece occurs when the latter is employed in a smaller quantity than the ratio of 1 : 1. He found that the heated as well as the fresh serum can furnish an active mid-piece, but a prolonged heating of the mid-piece at 55° C. finally destroys its function.

Liefmann studied the relation of certain lipoids and soaps to the mid- and end-pieces of complement and found that they have no effect upon the end-piece, but that they destroy the mid-piece. He brought out also the fact that persensitization can not take place unless the amount of amboceptor reaches nearly fifty units.

That the active principle of complement undergoes an inactive modification in the presence of various acids, alkalis, and salts has been carefully studied by Noguchi, von Liebermann, Hektoen, and others. According to the experiments of Noguchi, the complement is permanently injured by a strong solution of these substances, especially by the acids and alkalis, but when used within a certain limit, the activity can be restored with but slight loss of power.

EXPERIMENTAL PART.

There are at least five different procedures by which it is claimed that complement may be split[1] into two components. Sachs employs hydrochloric acid; Liefmann, carbon dioxid gas; Ferrata, dialyzes against distilled water; Michaelis and Skwirsky use a mixture of acid and alkaline phosphates; the fifth method is that of splitting by complement fixation.

[1] "Complement-splitting" is the term generally adopted by English speaking bacteriologists. This is the reason for the employment of the term in this paper, although a better word might be substituted, and the following work almost necessitates its replacement by the term "inactivation," since this is what has really been proved to take place in almost all cases of so-called splitting.

In our first paper we shall consider only the first three methods. In these the globulin fraction of the serum is precipitated during the process of splitting.

COMPLEMENT-SPLITTING WITH HYDROCHLORIC ACID.

The procedure introduced by Sachs is as follows: One cubic centimeter of guinea pig serum (complement) is mixed with 8.2 cubic centimeters of N/250 hydrochloric acid solution (in distilled water), and after standing at room temperature for one hour the mixture is centrifugalized, whereupon the precipitate settles down to the bottom leaving a clear supernatant fluid above. The supernatant fluid is carefully separated from the deposit[2] and is mixed with 0.8 of a cubic centimeter of N/25 sodium hydroxid solution (in 10 per cent. sodium chlorid). The addition of alkali is intended to neutralize the acid introduced into the serum for splitting, while the resultant solution is made isotonic at the same time (about 0.9 per cent. sodium chlorid). The deposit is washed with water and finally collected by centrifugalization, and then dissolved in 10 cubic centimeters of 0.9 per cent. sodium chlorid solution. The supernatant fluid and the precipitate, both now in clear solution, represent the end- and mid-piece, respectively. Thus, the supernatant fluid has no action upon the sensitized corpuscles unless the precipitate portion is added, and *vice versa*. Apparently the complement is separated into two components.

Experimental evidence was also brought forward to show that the end-piece or supernatant portion carries the labile zymotoxic component, and the mid-piece, or precipitate portion, possesses the property of enabling the end-piece to act upon corpuscles sensitized by amboceptor, and that it is not destroyed by the temperature of 56° C.

The above experiments have been confirmed by various workers, but we have been able to corroborate the results in part only, inasmuch as the supernatant fluid was found to be active upon sensitized corpuscles in most of our experiments (table I, experiment II) and was very seldom inactive.

[2] Finding that the supernatant fluid was often active without the addition of mid-piece, we took the precaution of filtering the end-piece through a hardened filter paper in order to be certain of the absence of the mid-piece.

TABLE I.

Complement-Splitting by Hydrochloric Acid.

Experiment I (Ideal Case).[3]

Amounts taken.	Guinea pig serum 1 c.c. + N/250 HCl 8.2 c.c. (in distilled water). Centrifugalized after 1 hr. at room temperature.			Guinea pig serum 1 c.c. + 0.9 % NaCl 9 c.c.
	Supernatant fluid + 0.8 c.c. N/25 NaOH in 10 per cent. NaCl.	Deposit dissolved in 10 c.c. of 0.9 per cent. NaCl.	Equal amounts of both ingredients.	After standing 1 hr. at room temperature (control).
	2 units of amboceptor and 0.1 c.c. of a 10 % suspension of corpuscles and the total volume made up to 15 c.c. Results taken after 1 hr.			
1.0 c.c.	No H.[4]	No H.	——	
0.7 c.c.	No H.	No H.	——	
0.5 c.c.	No H.	No H.	C.H.	
0.4 c.c.			C.H.	
0.3 c.c.			C.H.	
0.2 c.c.			Sl.H.	C.H.
0.15 c.c.			Tr.H.	F.C.H.
0.1 c.c.			No H.	Tr.H.

Experiment II (Usual Splitting).

Amounts taken.	Guinea pig serum 1 c.c. + N/250 HCl 8.2 c.c. (in distilled water). Centrifugalized after 1 hr. at room temperature.						Guinea pig serum 1 c.c. + 0.9 % NaCl 9 c.c.	
	Supernatant fluid + 0.8 c.c. N/25 NaOH in 10% NaCl.		Deposit dissolved in 10 c.c. of 0.9 % NaCl.		Equal volumes of both ingredients.		After standing at room temperature for 1 hr. (control).	
	2 units of amboceptor and 0.1 c.c. of a 10 % suspension of corpuscles added to each tube and the total volume made up to 1.5 c.c. Results taken after 30 and 60 min.							
	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
1.0 c.c.	C.H.		No H.	No H.				
0.7 c.c.	C.H.		No H.	No H.	C.H.	C.H.		
0.5 c.c.	F.C.H.	C.H.			C.H.	C.H.		
0.4 c.c.	Mch.H.	C.H.			C.H.	C.H.		
0.3 c.c.	Sl.H.	C.H.			C.H.	C.H.		
0.2 c.c.	No H.	Sl.H.			F.C.H.	C.H.	C.H.	C.H.
0.15 c.c.		No H.			No H.	Sl.H.	C.H.	C.H.
0.1 c.c.						No H.	Sl.H.	Mch.H.
0.07 c.c.							No H.	No H.

[3] Throughout this work 0.9 per cent. sodium chlorid solution was used instead of 0.85 per cent. usually employed by other workers.

[4] In this and the following tables, for the sake of brevity, certain symbols are employed to indicate the degree of hemolysis; thus C.H. = complete hemolysis; F.C.H. = fairly complete hemolysis; Sl.H. = slight hemolysis; Tr.H. = trace of hemolysis; No H. = no hemolysis; Mch.H. = much hemolysis.

TABLE II.

The Effect upon Components of Guinea Pig Serum of Heating for 30 Minutes at 56° C.

	Experiment I.[5]			*Experiment II.*			
	Fresh guinea pig serum 1 c.c.+8.2 c.c. HCl N/250 (in distilled water).			Guinea pig serum heated at 56° C. for 30 minutes + 8.2 c.c. HCl N/250 (in distilled water).			
	Kept at room temperature for 60 minutes, centrifugalized and supernatant fluid separated, neutralized, and made isotonic by 0.8 c.c. of N/25 NaOH in 10% NaCl.			Kept at room temperature for 60 minutes, centrifugalized and supernatant fluid separated, neutralized, and made isotonic by 0.8 c.c. of N/25 NaOH in 10% NaCl.			
	0.15 c.c. of supernatant fluid, 2 units of amboceptor, and 0.1 c.c. of 10% suspension of corpuscles in each tube.			0.2 c.c. of supernatant fluid, 2 units of amboceptor, and 0.1 c.c. of 10% suspension of corpuscles in each tube.			
	Results taken after :			Results taken after :			
	30 min.	60 min.	90 min.	30 min.	60 min.	90 min.	0 control.
+0	No H.	Sl.H.	C.H.	No H.	No H.	No H.	
+Alanin 0.3 c.c. of 7.5% solution in 0.9% NaCl.	C.H.	C.H.	C.H.	No H.	No H.	No H.	No H.
+Deposit from splitting in experiment I dissolved in 10 c.c. 0.9% salt solution. 0.15 c.c.	C.H.	C.H.	C.H.	No H.	No H.	No H.	No H.
+Deposit from splitting in experiment I dissolved in 10 c.c. 0.9% salt solution. 0.07 c.c.	C. H.	C.H.	C.H.	No H.	No H.	No H.	
+Deposit from splitting in experiment II dissolved in 10 c.c. 0.9% salt solution. 0.2 c.c.	No H.	Sl.H.	C.H.	No H.	No H.	No H.	No H.
+Deposit from splitting in experiment II dissolved in 10 c.c. 0.9% salt solution. 0.1 c.c.	C.H.[6]	C.H.	C.H.	No H.	No. H	No H.	

While the phenomenon suggests a double constitution of the complement, yet it does not exclude the possibility of its being caused by an excess of acid or alkali. Guinea pig serum, when mixed with hydrochloric acid in the proportion prescribed in this procedure, becomes almost neutral or but faintly alkaline. This causes the precipitation of certain proteids, mainly the globulins. Thus the acid introduced is quickly taken up by certain alkaline salts of the serum and there is none left free. The precipitated globulins also bind a certain amount of the acid. The supernatant fluid which is almost neutral and contains chiefly the albumin fraction of the serum, is then mixed with sufficient free alkali to neutralize exactly

[5] The total volume was brought up to 1 c.c. by 0.9 per cent. salt solution in both experiments.

[6] This phenomenon does not occur constantly. With some specimens of complement (very few) the mid-piece is undoubtedly destroyed by heating the whole serum previous to the splitting, but it is easier to destroy the mid-piece after its separation.

the amount of acid added, but this amount of alkali is in excess when added to the serum mixture from which the globulin fraction was removed after its precipitation by hydrochloric acid. Thus it happens that in the serum treated with the acid and then neutralized with the alkali in the manner prescribed, in the presence of the globulin fraction, the complement action is restored. On the other hand, if the globulin fraction is first removed and the same amount of the alkali is added to the remaining supernatant fluid, the complement activity of the supernatant fluid is paralyzed. The exami-

TABLE III.

Demonstration of Complement Inactivation by Sodium Hydroxid in the Process of Complement-Splitting.

	Tubes.					
	1	2	3	4	5	6
Guinea pig serum.....	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.
N/250 HCl.....	8.2 c.c.	8.2 c.c.	8.2 c.c.	8.2 c.c.	———	———
0.9% NaCl.....					9.0 c.c.	
Distilled water.....						8.2 c.c.
After 1 hour at room temperature all the tubes were centrifugalized. From tubes 1 and 2 only supernatant fluid was used; from the others the entire contents of the tubes were used.						
N/25 NaOH in 10% NaCl.....	0.8 c.c.	———	———	0.8 c.c.	———	———
10% NaCl.....	———	0.8 c.c.	0.8 c.c.	———	———	0.8 c.c.
	Supernatant fluid		The entire contents of the tubes			
Reaction of resulting fluids to alizarin-indigo[7] (calculated for the whole volume).	5.6 c.c. HCl N/250	2.5 c.c. NaOH N/250	2.5 c.c. NaOH N/250	5.6 c.c. HCl N/250	6.2 c.c. HCl N/250	6.2 c.c. HCl N/250
Complement activity of the resulting mixtures (with 0.1 c.c. of 10% suspension of corpuscles and 2 units of amboceptor.						
Amounts of mixture. 1.0 c.c.	C.H.					No H.
0.7 c.c.	Sl.H.					
0.5 c.c.	Tr.H.			C.H.		
0.3 c.c.	No H.	C.H.	C.H.	F.C.H.		
0.2 c.c.		F.C.H.	F.C.H.	Mch.H.	C.H.	
0.15 c.c.		Mch.H.	Sl.H.	Tr.H.	F.C.H.	
0.1 c.c.		No H.	No H.	No H.	Sl.H.	
0.07 c.c.					No H.	

[7] The use of this indicator, first introduced by Bernhardt, was suggested to us by Prof. W. J. Gies. The indicator is not noticeably influenced by the presence of proteids or carbon dioxid.

nation of the so-called neutralized supernatant fluid for alkalinity indicates a slight excess of alkali over that present in the same dilution of the untreated serum.

That this slight excess of alkalinity is not solely responsible for the loss of action of the untreated complement, but that the nature of the alkali employed has more to do in paralyzing the complement seems very probable. Thus, the free alkali in the form of sodium hydroxid fails to restore the original alkaline salts, and exerts an injurious effect upon the active complement constituents. That this is the case is easily shown by the experiments in table III.

These experiments show that the supernatant fluid from which the native alkalinity has been almost completely removed by hydrochloric acid still has nearly two thirds of the original complement power of the serum. But by adding gradually increasing amounts of sodium hydroxid, the activity is steadily reduced (table V) until in some cases it disappears completely (table VI), when the amount of sodium hydroxid reaches the point supposed to be necessary to restore the original alkalinity of the treated serum. The completeness of the inactivation of the complement action of the supernatant fluid is also enhanced by a slight excess of the free sodium hydroxid toward which the complement shows a great susceptibility. It may be assumed that the salts that cause the native alkalinity, principally the carbonates and phosphates, are split by hydrochloric acid and form sodium chlorid and the acids, but the greater part of carbon dioxid leaves the fluid, thus causing the free alkali introduced to act directly upon the complement.

It is interesting, however, that, when the serum was acted on by hydrochloric acid and sodium hydroxid was subsequently added, without removing the globulin fraction, much if not all of the complement activity was restored (table IV, A).

The reason for sodium hydroxid not causing the same injurious effect upon the complement in this instance (table IV, A) must be due to the presence of the globulin fraction which seems to have a greater affinity for the free sodium hydroxid than the albumin fraction in which the active principle of the complement seems to reside.

That the globulin fraction has a greater affinity for sodium

TABLE IV.

Comparison of Complement Inactivation by Different Alkalis in the Presence and Absence of Globulin Fractions.

Amounts of neutralized dilution used.	To 1 c.c. of guinea pig complement 8.2 c.c. of N/250 HCl were added and kept at room temperature for 1 hr.								1 c.c. complement + 8.2 c.c. HCl N/250 in 0.9% NaCl.		1 c.c. complement + 9 c.c. 0.9% NaCl.	1 c.c. complement + 8.2 c.c. distilled water.
	After 1 hr. corresponding amounts of alkali were added to neutralize the acidity and made isotonic.						Made isotonic without neutralization (control).		Neutralized with NaOH.	Non-neutralized.		Made isotonic.
	NaOH		$NaHCO_3$		Na_2HPO_4							
	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Whole serum.		
1.0 c.c.		C.H. Sl.H. No H.		C.H. Mch.H. Sl.H. No H.		C.H. Sl.H. No H.		C.H. F.C.H. No H.	Mch.H. Sl.H. No H.			No H.
0.7 c.c.												
0.5 c.c.												
0.3 c.c.					C.H. Sl.H. No H.		C.H. F.C.H. No H.					
0.2 c.c.	C.H. No H.		C.H. F.C.H. Sl.H. No H.							C.H. Mch.H. Sl.H. No H.	C.H. Sl.H. No H.	
0.15 c.c.												
0.1 c.c.												
0.07 c.c.												
	A		B		C		D		E		F	G

TABLE V.

The Effect of the Excess of Hydrochloric Acid and Sodium Hydroxid upon the Serum from Which the Globulin Fraction Is Removed.

1 c.c. of complement + 8.2 c.c. of N/250 HCl at room temperature for 1 hour, then centrifugalized and the globulin fraction removed. Only the supernatant fluid was used after it was made isotonic by 10 per cent. NaCl.

Amounts of mixtures.	1 c.c. of supernatant fluid in all tubes, then different amounts of HCl or NaOH were added and the resultant mixtures tested in varying amounts.								
	N/250 HCl				Original isotonized supernatant fluid. 0 (non-neutralized.)	N/250 NaOH.			Native complement + 1 c.c. 0.9% NaCl 0 c.c.
	0.8 c.c.	0.6 c.c.	0.4 c.c.	0.2 c.c.		0.2 c.c.	0.4 c.c.	0.6 c.c.	0.8 c.c.	
0.5 c.c.	No H	Mch.H.							Sl.H.(?)	
0.3 c.c.	No H.	F.C.H.	C.H.				C.H.	C.H.	C.H.	
0.2 c.c.	Sl.H.	C.H.	F.C.H.(?)	C.H.			F.C.H.	F.C.H.	F.C.H.	
0.1 c.c.	Sl.H.	C.H.	F.C.H.(?)	F.C.H.(?)			F.C.H.	F.C.H.	F.C.H.	
0.07 c.c.	Tr.H.	Sl.H.	F.C.H.(?)	F.C.H.(?)	C.H.	C.H.	F.C.H.	Sl.H.	Sl.H.	
0.05 c.c.	Tr.H.(?)	Tr.H.	Mch.H.	F.C.H.(?)	F.C.H.(?)	F.C.H.	Sl.H.	Sl.H.	Sl.H.	
0.03 c.c.	No H.	No H.[8]	Sl.H.	Sl.H.	F.C.H.(?)	Sl.H.	Sl.H.	Sl.H.	No H.[8]	
0.02 c.c.			No H.	No H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	No H.	C.H.
0.01 c.c.					No H.	No H.	Tr.H.(?)	Sl.H.	No H.	F.C.H.
0.005 c.c.							No H.	No H.		No H.

[8] So-called "Endstück."

hydroxid than the albumin fraction is easily demonstrated by experiments described later.

In order to ascertain whether the loss of complement action in the albumin fraction, the so-called end-piece, is really due to a reversible inactivation through the action of sodium hydroxid, such as had been observed by Noguchi in his earlier experiments, and not due to the removal of one of the interdependent components, the mid-piece, it was necessary to make a series of reverse experiments with an acid.

The following experiments show that the complement activity of the inactive end-piece can be restored to a great extent by means of neutralization of the alkali with adequate amounts of hydrochloric acid (table VII).

We now come to consider the mechanism of the restoration of the complement activity of the end-piece by the globulin fraction known as the mid-piece. From the reactivation experiments with hydrochloric acid, it is not improbable that the combination of the complement and sodium hydroxid can also be split by means of an amphoteric compound. Among the amphoteric substances we find various proteids, proteoses, and many non-coagulable synthetic amino acids. The serum globulins are undoubtedly amphoteric.

In the present experiments we have also used egg-white, alanin, deuteroalbumose, and peptone.[9]

These experiments (table VII) demonstrate that the complement action of the so-called end-piece can be readily restored by adding adequate quantities of the amphoteric substances herein employed. One may wonder how such a weak acid as the amino acid, which does not form a salt at ordinary temperature, can reverse the combination of the alkali and the complement fraction. But, it must be remembered that the affinity between the complement and alkali cannot be stronger than that which exists between any other amphoteric bodies. Moreover, the direction of the reversion is dependent upon the total number of acid radicals possessed by a given substance. Thus, a greater amount of the substance is

[9] We are indebted to Dr. F. J. Birchard, of The Rockefeller Institute for Medical Research, for the albumose and peptone preparations. The albumose was prepared from fibrin according to Pick's method, and the peptone from Witte's peptone according to Sigfried's method.

	NaOH N/25 in c.c.	Amounts of 0.9 % NaCl and 3 % alanin in c.c.	Supernatant fluid (after centrifugalization) of the mixture. 1 c.c. guinea pig serum + 8.2 c.c. HCl N/250 for 1 hour at room temperature.							
			1 c.c.	0.7 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.
Amounts of NaOH N/25 in 10% NaCl added to each 1 c.c. of the solutions examined.	0	Salt 0.3			C.H.	F.C.H.	Sl.H.	Sl.H.	No H.	
		Alanin 0.3					C.H.	C.H.	Mch.H.	No H.
	0.01	Salt 0.3					C.H.	Sl.H.	No H.	
		Alanin 0.3						C.H.	No H.	
	0.015	Salt 0.3				C.H.	Mch.H.	No H		
		Alanin 0.3					C.H.	F.C.H.	F.C.H.	No H.
	0.02	Salt 0.3				C.H.	F.C.H.	Tr.H.	No H.	
		Alanin 0.3					C.H.	Tr.H.	No H.	
	0.03	Salt 0.3				C.H.	Tr.H.	No H.		
		Alanin 0.3				C.H.	F.C.H.	No H.		
	0.04	Salt 0.3	C.H.	F.C.H.	No. H.					
		Alanin 0.3			C.H.	F.C.H.	F.C.H.	No H.		
	0.05	Salt 0.3	C.H.	F.C.H.	No H.					
		Alanin 0.3			C.H.	F.C.H.	Sl.H.	No H.		
	0.07[9]	Salt 0.3	No H.							
		Alanin 0.3				C.H.	Tr.H.	No H.		
	0.1	Salt 0.3	No H.							
		Alanin 0.3			C.H.	F.C.H.	Sl.H.	No H.		

	NaOH N/25 in c.c.	Amounts of 0.9 % NaCl and 3 % alanin in c.c.	Guinea pig complement 1 c.c. + 9 c.c. 0.9% NaCl for 1 hour at room temperature (control).								
			1 c.c.	0.7 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.	0.05 c.c.
Amounts of NaOH N/25 in 10% NaCl added to each 1 c.c. of the solutions examined.	0	Salt 0.3							C.H.	F.C.H.	F.C.H.
		Alanin 0.3									
	0.01	Salt 0.3									C.H.
		Alanin 0.3									C.H.
	0.015	Salt 0.3									F.C.H.
		Alanin 0.3									C.H.
	0.02	Salt 0.3								F.C.H.	Sl.H.
		Alanin 0.3								C.H.	C.H.
	0.03	Salt 0.3							Sl.H.	No H.	
		Alanin 0.3							C.H.	Tr.H.	No H.
	0.04	Salt 0.3				C.H.	F.C.H.	Mch.H.	Sl.H.	No H.	
		Alanin 0.3					C.H.	Mch.H.	Sl.H.	No H.	
	0.05	Salt 0.3			C.H.	F.C.H.	No H.				
		Alanin 0.3				C.H.	F.C.H.	No H.			
	0.07[10]	Salt 0.3	C.H.	Sl.H.	No H.						
		Alanin 0.3			C.H.	F.C.H.	No H.				
	0.1	Salt 0.3	No. H.								
		Alanin 0.3			C.H.	F.C.H.	Tr.H.(?)	No H.			

In this experiment sheep corpuscles were used (0.5 c.c. of 5 per cent. suspension) and 50 units of amboceptor.

[10] The amount of sodium hydroxid used in the original complement-splitting experiments corresponds to 0.08 c.c. in this series.

TABLE VII.

Reactivation of the End-Piece of the Complement Split by Hydrochloric Acid.

	Alanin (5 %), 0.075 c.c.	α peptone (2 %), 0.1 c.c.	Deutero-albumose B (2 %), 0.05 c.c.	Mid-piece from HCl-splitting, 0.05 c.c.	Mid-piece + NaOH N/250 (1 c.c. + 0.25 c.c.), 0.05 c.c.	Mid-piece heated 30 min. at 56° C., 0.05 c.c.	Sheep serum diluted 1:10 heated 56° C., 0.05 c.c.	Mid-piece from dialysis, 0.05 c.c.	Mid-piece from dialysis + HCl ⅓ (3:1), 0.05 c.c.	HCl N/250	o control.
Native complement 0.03 c.c. inactivated by 0.6 c.c. NaOH N/250	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	0.005 c.c. C.H.	No H.
End-piece[11] non-neutralized, 0.02 c.c.	No H.	No H.	Sl.H.	Sl.H.	F.C.H.	F.C.H.	Mch.H.	Mch.H.	Tr.H.	0.005 c.c. No H.	Tr.H.(?)
End-piece[12] 9.2 c.c. + 0.2 NaOH N/25 in NaCl, 0.05 c.c.	Sl.H.	Tr.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Tr.H.(?) Sl.H.(?)
End-piece[12] 9.2 c.c. + 0.4 c.c. NaOH N/25 in NaCl, 0.05 c.c.	Mch.H.	F.C.H.	C.H.	F.C.H.	F.C.H.	Sl.H.	Tr.H.	F.C.H.	F.C.H.	0.02 c.c. F.C.H.	
End-piece[12] 9.2 c.c. + 0.8 c.c. NaOH N/25 in NaCl, 0.05 c.c.	Mch.H.	F.C.H.	C.H.	Sl.H.	Mch.H.	F.C.H.	Tr.H.	Sl.H.	Sl.H.	0.04 c.c. C.H.	Tr.H.
End-piece[12] 9.2 c.c. + 0.4 c.c. HCl N/25, 0.03 c.c.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Mch.H.	Tr.H.	Tr.H.	0.005 c.c. Tr.H.	Tr.H.
Control 0	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	

[11] The end-piece resulted from mixing 1 c.c. of complement + 8.2 c.c. N/250 HCl at room temperature for 60 minutes. Then the mixture was centrifugalized and the supernatant fluid made isotonic and diluted to 10 c.c.

[12] All the mixtures, beginning with the third end-piece, before being made isotonic were neutralized (partly) by the amounts of NaOH shown, then made isotonic and, if necessary, diluted to 10 c.c.

required if it possesses fewer acid radicals. That the globulins have a greater affinity for sodium hydroxid than the albumins may be accounted for by the larger size of the molecules of the former class of proteids. The solubility of the substances seems also to be important, as certain insoluble amino acids, such as asparagin, asparagin acid, and leucin failed to reactivate the end-piece.

COMPLEMENT-SPLITTING WITH CARBON DIOXID.

Liefmann splits the complement in the following manner. One half of a cubic centimeter of guinea pig serum (complement) is mixed with two cubic centimeters of distilled water, and carbon dioxid gas is passed through until no more precipitate is formed. The treated serum is then centrifugalized to separate the precipitate from the supernatant fluid. The clear supernatant fluid is carefully decanted and filtered through hardened filter paper into another receptacle and is used as the end-piece after being made isotonic with sodium chlorid. The deposit, which represents the globulin fraction of the serum, is then dissolved in 2.5 cubic centimeters of 0.85 per cent. salt solution. This portion is used as the mid-piece. In a certain number of instances the phenomenon of splitting is ideally shown, the end- and mid-pieces separately having no action upon the sensitized corpuscles (table X). The two components obtained by the carbon dioxid method behave in much the same way as those prepared by the hydrochloric acid method of Sachs. It may be stated, however, that the end-piece portion remains quite active in many instances, as has been observed also by Liefmann and others (tables IX and X).

In considering the processes that lead to the inactivation of the end-piece by carbon dioxid and the subsequent reactivation of this portion by the mid-piece of the globulin fraction, one can conceive that an essential difference exists between the inactivation by carbon dioxid and that by the hydrochloric acid method. In case of the hydrochloric acid-splitting, the carbonates as well as other alkaline salts of the serum are completely converted into chlorids. Small amounts of free carbon dioxid or other weak acids remaining in the fluid cause a partial inactivation of the complement.

On the other hand, the passage of carbon dioxid through a

TABLE VIII.

Inactivation of Complement by Carbon Dioxid.

Dilution 1:10 in water.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.04 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.005 c.c.
Native complement in NaCl									C.H.	F.C.H.	No H.
Complement + CO_2, 2 min. (whole)						C.H.	Tr.H.	No H.			
Complement + CO_2, 10 min. (made isotonic)				C.H.	F.C.H.	No H.	No H.				
Supernatant fluid alone											
Guinea pig complement after passage of CO_2, 2 min.				C.H.	Sl.H.	No H.					
Guinea pig complement after passage of CO_2, 4 min.			C.H.	F.C.H.	F.C.H.	Tr.H.(?)	No H.				
Guinea pig complement after passage of CO_2, 6 min.			C.H.	Tr.H.	No H.						
Guinea pig complement after passage of CO_2, 8 min.		C.H.	Mch.H.	No H.							
Guinea pig complement after passage of CO_2, 10 min.	C.H.	F.C.H.	Mch.H.	No H.							

TABLE IX.

Splitting by Carbon Dioxid.

	Amount of end and mid-piece.							
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.
Native complement 1:10				C.H.	F.C.H.	Mch.H.	Tr.H.	No H.
1:10 complement (CO_2), 10 min. (whole)				C.H.	Mch.H.	Sl.H.	No H.	
Albumin fraction, CO_2, 10 min. (end-piece)	C.H.	F.C.H.	Mch.H.	Tr.H.(?)	No H.			
End-piece[13] (CO_2), 10 min. +mid-piece, 10 min. 1:1			C.H.	F.C.H.	F.C.H.	Tr.H.	No H.	
End-piece (CO_2), 10 min. +mid-piece, 60 min. 1:1,		C.H.	F.C.H.	Mch.H.	No H.			
Albumin fraction (CO_2), 60 min. (end-piece)			C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
End-piece, 60 min., +mid-piece, 10 min., 1:1	F.C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.(?)	No H.		
End-piece, 60 min., +mid-piece, 60 min., 1:1			C.H.	F.C.H.	Mch.H.	No H.		
Mid-piece, 10 min., alone	No H.							
Mid-piece, 60 min., alone	No H.							

[13] 1:1 means equal amounts of both end- and mid-pieces; therefore in the column under 0.2 c.c. it means 0.2 c.c. of end-piece +0.2 c.c. of mid-piece.

TABLE X.

A Case of Successful Splitting by Carbon Dioxid (Very Rare).

	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.
Native complement 1:10 dilution							C.H.	Sl.H.	No H.	
Complement + CO_2, 10 min., made isotonic (whole)							C.H. (delay)	Sl.H.	No H.	
Albumin fractions alone	No H.									
Albumin fraction + globulin fraction 1:5					C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
Globulin fraction alone	No H.									

A Case of Imperfect Splitting by Carbon Dioxid.

	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c.c.
Native complement 1:10 dilution				C.H.	F.C.H.(?)	F.C.H.	Sl.H.	No H.
Complement + CO_2, 10 min., made isotonic (whole)				C.H.	F.C.H.	Mch.H.	No H.	
Albumin fraction alone				C.H.	Mch.H.	Sl.H.	No H.	
Albumin fraction + globulin fraction 1:5	C.H.	F.C.H.	Sl.H.	Sl.H.	No H.			

A Case of the Complement Twenty-four Hours Old.

	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c c.
Native complement 1:10 dilution	C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	
Complement CO_2, 10 min., made isotonic (whole)		C.H.	F.C.H.	Mch.H.	Tr.H.	No H.	
Albumin fraction alone			C.H.	Mch.H.	Sl.H.	Tr.H.	No H.
Albumin fraction + globulin fraction 1:1	C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	

TABLE XI.

Inactivation of Complement by Carbon Dioxid in Different Media.

	+ CO_2, 10 min.						
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c	0.07 c. c.	0.05 c.c.	0.03 c. c.
Native complement 1:10 in NaCl							
Complement 1 c.c. +8.2 c.c. distilled water, isotonized after 30 min.			C.H.	F.C.H.	Sl.H.	Tr.H.	No H.
Complement 1 c.c. +8.2 c.c. 0.9% NaCl, after 30 min. 0.8 c.c. 0.9% NaCl added					C.H.	Sl.H.	No H.
Complement 1 c.c. +8.2 c.c. N/250 HCl (in distilled water), neutralized and made isotonic after 30 min.		C.H.	Mch.H.	Mch.H.	Sl.H.	Tr.H.	No H.
Complement 1 c.c. + 8.2 c.c. HCl in 0.9% NaCl, neutralized after 30 min.				C.H.	Mch.H.	Sl.H.	Tr.H.
Complement[14] in isotonic sugar solution 1:10 +0.3 c.c. NaCl 0.9%	C.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	No H.	

	No CO_2.							
	c.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.
Native complement 1:10 in NaCl						C.H.	F.C.H.	No H.
Complement 1 c.c. +8.2 c.c. distilled water, isotonized after 30 min.	Tr.H.	No H.						
Complement 1 c.c. +8.2 c.c. 0.9% NaCl, after 30 min. 0.8 c.c. 0.9% NaCl added					C.H.	C.H.	F.C.H.	No H.
Complement 1 c.c. +8.2 c.c. N/250 HCl (in distilled water) neutralized and made isotonic after 30 min.		C.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	No H.	
Complement 1 c.c. +8.2 c.c. HCl in 0.9% NaCl, neutralized after 30 min.		C.H.	C.H.	C.H.	F.C.H.	Sl.H.	Tr.H.	No H.
Complement[14] in isotonic sugar solution 1:10 +0.3 c.c. NaCl 0.9%						C.H.	Sl.H.	No H.

[14] The addition of sodium chlorid is to prevent complement from complete inactivation.

TABLE XII.

Reactivation of Complement Inactivated by Carbon Dioxid.

Amounts of reactivating substances.	Alanin 3 %.	Mid-piece (CO_2).	Egg-white 1:10.	Sheep serum, 30 min. at 56° C. 1:5.	Protoalbumoses 5 %.	Deuteroalbumoses 2 %.
0.3 c.c. of end-piece from CO_2-splitting in each tube. 1.0 c.c.						
0.7 c.c.						
0.5 c.c.						
0.4 c.c.	C.H.					
0.3 c.c.	C.H.	Sl.H.				
0.2 c.c.	C.H.	Mch.H.				
0.15 c.c.	F.C.H.	Mch.H.			No. H.	
0.1 c.c.	Mch.H.	C.H.		Sl.H.	Sl.H.	No. H.
0.05 c.c.		Mch.H.		Sl.H.	Mch.H.	Tr.H.
0.03 c.c.		Sl.H.	C.H.	F.C.H.	Sl.H.	Mch.H.
0.02 c.c.			C.H.			Sl.H.
0.01 c.c.			C.H.			
0.005 c.c.			C.H.			
0						

mixture of guinea pig serum and water precipitates the globulin fraction just as in the case of the hydrochloric acid treatment, but the carbonates as well as phosphates remain unaffected by this treatment. The inactivation of the complement in this instance is doubtless due to the overcharging of the serum water with carbon dioxid gas, which by virtue of its acid nature combines with the amphoteric molecules of the serum albumin fraction in which the activity of the complement is residing. In a medium, whether in the undiluted native serum or in a serum diluted with sodium chlorid solution of isotonic concentration, in which the sodium chlorid content reaches a sufficient concentration (table XI), a weak acid like carbon dioxid fails to enter into even a loose combination with the albumin molecules, and hence leaves the complement comparatively uninjured. The reason that carbon dioxid acts upon the complement molecules in serum sufficiently diluted with water is that the strong electrolyte, sodium chlorid, can in that dilution no longer interfere with the action of a weak acid. In diluted serum carbon dioxid causes an acid inactivation of complement and the activity of complement may be restored by certain amphoteric substances (table XII) and also by an alkali (table XV, C), but not by an acid.

The phenomenon of reactivation by means of adequate quantities of sodium hydroxid may raise a question as to why the result-

TABLE XIII.

Substances Subjected to Carbon Dioxid Lose Their Reactivating Power for Complement Inactivated by Carbon Dioxid.

Substances acted upon by carbon dioxid for 15 minutes. The total volume in each tube was 1.5 c.c.

	Alanin 5%.				Mid-piece (CO_2).				o Control.
	No CO_2.		+ CO_2		No CO_2.		+ CO_2.		
	0.15 c.c.	0.1 c.c.	0.15 c.c.	0.1 c.c.	0.07 c.c.	0.03 c.c.	0.07 c.c.	0.03 c.c.	
End-piece (CO_2) 0.07 c.c. in all tubes.	C.H.	C.H.	No H.	Tr.H.	C.H.	C.H.	Tr.H.	Sl.H.(?)	No H.
No end-piece (control)	No H.		No H.		No H.		No H.		

	Egg-white (1:10).				Sheep serum 56° C. (1:5).				o Control.
	No CO_2.		+ CO_2.		No CO_2.		+ CO_2.		
	0.01 c.c.	0.005 c.c.	0.01 c.c.	0.005 c.c.	0.03 c.c.	0.01 c.c.	0.03 c.c.	0.01 c.c.	
End-piece (CO_2) 0.07 c.c. in all tubes.	C.H.	C.H.	Tr.H.	Tr.H.	C.H.	F.C.H.	Tr.H.	Mch.H.	No H.
No end-piece (control)	No H.		No H.		No H.		No H.		

ant Na_2CO_3 or $NaHCO_3$ no longer exerts the same effect as the free sodium hydroxid and carbon dioxid, since these are readily dissociable in the fluid. But one must not forget that in the stage of reactivation the fluid is now made isotonic with sodium chlorid, and this restrains the dissociation of carbonates or phosphates sufficiently to prevent the free action of the carbon dioxid or sodium ions upon the complement fraction. It is important to notice that an acid has no reactivating property for the end-piece prepared with carbon dioxid, while exactly the opposite is the case with the end-piece made with hydrochloric acid, which is an inactivation by alkali.

In the experiments in table XIII we show that amphoteric substances capable of reactivating complement inactivated by carbon dioxid lose this reactivating property if treated with carbon dioxid before being added to the inactive complement. The way in which amphoteric substances reactivate complement is thus clearly demonstrated.

COMPLEMENT-SPLITTING BY MEANS OF DIALYSIS.

Before Sachs, Liefmann, and others introduced various methods of splitting the complement, Ferrata discovered that by dialyzing complement against water through a fish membrane or a celloidin sac, it could be separated into two inactive components, an albumin fraction corresponding with the end-piece of the later investigators, and a globulin fraction (which precipitates during dialysis) corresponding with the mid-piece. This result, although not always obtained, was nevertheless confirmed by other workers.

On several occasions in the present series of experiments we have also confirmed this finding. Our explanation for this phenomenon is, however, entirely different from that given by previous workers. As will be shown in the following protocols, the supernatant fluid (albumin fraction) when separated from the precipitate (globulin fraction) and made isotonic with sodium chlorid, is quite inactive upon the sensitized corpuscles, but can be reactivated by adding, not only the globulin fraction of the same serum (isotonized with sodium chlorid), but also the globulin fraction prepared by the hydrochloric acid or carbon dioxid methods (table XIV). Indeed,

TABLE XIV.

Reactivation of Complement Inactivated by Distilled Water or Dialysis.[15]

	Alanin 5 %.			Mid-piece (CO_2, 10 min.).			Mid-piece dialysis.		
	0.3 c.c.	0.15 c.c.	0.07 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.
Complement + distilled water (1:10) 2 hours at 37° C., whole made isotonic (NaCl) ..... 0.2 c.c.	C.H.	Sl.H.	No H.	Mch.H.	C.H.	F.C.H.	No H.	No H.	No H.
Complement dialyzed at room temperature 6 hours. *End-piece* isotonized (final dilution 1:10) ..... 0.2 c.c.	C.H.	F.C.H.	No H.	F.C.H.	C.H.	F.C.H.	No H.	No H.	Sl.H.
End-piece (CO_2) (final dilution 1:10) (control) ..... 0.2 c.c.	C.H.	F.C.H.	No H.	Sl.H.	F.C.H.	C.H.	Sl.H.	F.C.H.	No H.
No complement. Control	No H.			No H.			No H.		

	Egg-white (1:10).			Sheep serum (56° C.) 1:5.			Control No mid piece.
	0.1 c.c.	0.05 c.c.	0.03 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	
Complement + distilled water (1:10) 2 hours at 37° C., whole made isotonic (NaCl) ..... 0.2 c.c.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
Complement dialyzed at room temperature 6 hours. *End-piece* isotonized (final dilution 1:10) ..... 0.2 c.c.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
End-piece (CO_2) (final dilution 1:10) (control) ..... 0.2 c.c.	Tr.H.	Sl.H.	C.H.	C.H.	C.H.	C.H.	No H.
No complement. Control	No H.			No H.			

[15] Results taken after incubation at 37° C. for 1 hour.

the reactivation may be even better and more constant with mid-pieces prepared by these other methods than with its own mid-piece. Further, it is found that an acid (table XV, B) or certain amphoteric substances which bear no relation to the serum (table XIV)

TABLE XV.

Reactivation of Complement Inactivated by Acid (Carbon Dioxid) Compared to Reactivation of Complement by Alkali (Dialysis or Distilled Water).

	Incubation time.	Complement + distilled water (1:10) at room temperature for 1 hr.; the whole made isotonic; 0.5 c.c. used in each tube.					
		+ Alanin 7.5 %.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
A	HCl N/250 0.1		No H.	No. H.	Tr.H.	No. H.	Sl.H.
	HCl N/250 0.125		No H.	Tr.H.	Sl.H.	Sl.H.	F.C.H.
	HCl N/250 0.15		No H.	Sl.H.	Mch.H.	Sl.H.	F.C.H.
	Control	F.C.H.	F.C.H.	C.H.	C.H.	No H.	No H.(?)
	NaOH N/250 0.1						
	NaOH N/250 0.2						
	NaOH N/250 0.3	No H.	No H.	No H.	No. H.		
	NaOH N/250 0.4	No H.	Tr.H.(?)	No H.	Tr.H.	No H.	No H.
	NaOH N/250 0.5	Tr.H.	Tr.H.	Sl.H.(?)	Sl.H.	No H.	No H.

	Incubation time.	End-piece of complement dialyzed for 12 hrs. against running water; made isotonic; final dilution 1:10; 0.3 c.c. in each tube.					
		+ Alanin 7.5 %.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
B	HCl N/250 0.1		No H.	No H.	No H.	No H.	No H.
	HCl N/250 0.125		No H.	Sl.H.	Mch.H.	Sl.H.	Mch.H.
	HCl N/250 0.15		No H.	Sl.H.	Mch.H.	Sl.H.	F.C.H.
	Control	F.C.H.	C.H.	C.H.	C.H.	No H.	Tr.H.
	NaOH N/250 0.1						
	NaOH N/250 0.2						
	NaOH N/250 0.3		No H.		No H.		No H.
	NaOH N/250 0.4		No H.		No H.		No H.
	NaOH N/250 0.5		Tr.H.		Tr.H.		No H.

	Incubation time.	End-piece (CO_2) for 10 min.; made isotonic; final dilution 1:10; 0.3 c.c. in each tube.					
		+ Alanin 7.5 %.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
C	HCl N/250 0.1		No H.		No H.	No H.	Tr.H.
	HCl N/250 0.125		No H.		No H.	No H.	Tr.H.
	HCl N/250 0.15		No H.		No H.	No H.	Tr.H.
	Control	Sl.H.	C.H.	Tr.H.	C.H.	No H.	Sl.H.
	NaOH N/250 0.1	No H.	Tr.H.	No H.	Tr.H.		
	NaOH N/250 0.2	Tr.H.	F.C.H.	Tr.H.	C.H.	C.H.	
	NaOH N/250 0.3	Mch.H.	C.H.	Mch.H.	C.H.	C.H.	
	NaOH N/250 0.4						
	NaOH N/250 0.5						

reactivate this dialyzed end-piece. On the other hand, alkalis do not reactivate it (table XV, B).

From the experiments given above it becomes highly probable that dialyzed serum loses its complement activity chiefly by an alkali inactivation of a reversible character. The cause of this

TABLE XVI.

Rate of Dialysis of Sodium Chlorid and $NaHCO_3$ from the Mixture of Fifty Cubic Centimeters of 1 per cent. Sodium Chlorid + Fifty Cubic Centimeters N/50 $NaHCO_3$.

	NaCl				$NaHCO_3$			
	$AgNO_3$	NH_4SCN	Combined Ag	NaCl	H_2SO_4 N/100	NaOH N/100	Combined acid	$NaHCO_3$ N/50
After[16]								
0	4.0 c.c.	2.0 c.c.	2.0 c.c.	0.02 gm.	6.0 c.c.	1.0 c.c.	5.0 c.c.	2.5 c.c.
20 min.	3.0 c.c.	2.0 c.c.	1.0 c.c.	0.01 gm.	4.0 c.c.	1.0 c.c.	3.0 c.c.	1.5 c.c.
40 min.	2.0 c.c.	1.2 c.c.	0.8 c.c.	0.008 gm.	2.5 c.c.	0.5 c.c.	2.0 c.c.	1.0 c.c.
1 hr.	1.0 c.c.	0.6 c.c.	0.4 c.c.	0.004 gm.	2.5 c.c.	1.3 c.c.	1.2 c.c.	0.6 c.c.
2 hrs.	0.5 c.c.	0.4 c.c.	0.1 c.c.	0.001 gm.	2.0 c.c.	1.3 c.c.	0.7 c.c.	0.35 c.c.
3 hrs.	0.5 c.c.	0.5 c.c.	0	Trace(?)	1.5 c.c.	0.9 c.c.	0.6 c.c.	0.3 c.c.
4 hrs.	0.5 c.c.	0.5 c.c.	0	0	1.5 c.c.	1.0 c.c.	0.5 c.c.	0.25 c.c.
Running water	1.0 c.c.	1.0 c.c.	0	0	1.5 c.c.	1.0 c.c.	0.5 c.c.	0.25 c.c.

[16] At the time indicated, 5 c.c. of the contents of the collodion sac were removed and examined for hydrochloric acid and $NaHCO_3$.

inactivation may be found in the gradual changes taking place in the salt content of the serum within the sac during dialysis. As may be expected, sodium chlorid passes out of the celloidin sac with a greater velocity than the remaining salts, such as carbonates or phosphates, and this together with an increase in volume (by endosmosis of water) results in removing the restraining power of the chlorids upon the dissociation of the carbonates. These salts then inactivate the complement.

A study of the comparative velocity of osmosis of sodium chlorid and Na_2CO_3 through a celloidin membrane is given in table XVI. At the time indicated, five cubic centimeters of the contents of the collodion sac were removed and examined for sodium chlorid and sodium bicarbonate.

SUMMARY.

It is generally accepted that complement may be split into a mid-piece and an end-piece. The mid-piece is thought to be in the globulin fraction, and the end-piece in the albumin fraction. The restoration of complement activity by putting together the albumin and globulin fractions does not prove, however, that each fraction contained a part of the complement, for the albumin fraction can be reactivated in the absence of the globulin fraction.

Complement-splitting as brought about by hydrochloric acid, carbon dioxid, and dialysis, is really an inactivation of the whole complement by certain acids or alkalis, either added in the free state to the serum, or liberated as a result of the dissociation of certain electrolytes.

That the whole complement, and not a part only, is present in the albumin fraction of the serum can be demonstrated by the removal of the inhibitory action of the acid or alkali. This can be effected by the addition, not only of alkali or acid, but also of any amphoteric substance. When hydrochloric acid, carbon dioxid, or dialysis are employed to produce the phenomenon known as complement-splitting, the complement is merely inactivated, not split.

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[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XV, No. 6, 1912.]

A BIOCHEMICAL STUDY OF THE PHENOMENA KNOWN AS COMPLEMENT-SPLITTING.

SECOND PAPER: SPLITTING OF THE COMPLEMENT WITHOUT A VISIBLE ALTERATION OF THE PROTEID CONSTITUENTS.*

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In the preceding communication we have dealt with various phenomena of complement-splitting in which the globulin fraction of the serum was precipitated and subsequently separated from the albumin fraction. In all these processes, according to current conceptions, the globulin fractions contained the mid-piece of complement, and the albumin fractions, the end-piece. Yet, as has already been pointed out, the so-called end-piece of the albumin portion depends in no way upon the introduction of the mid-piece in order to regain its original complement property, but can be reactivated by any reagent carrying the ions opposite in sign to those keeping the active atom-complex of complement temporarily inactive. The precipitation of the globulin fraction, therefore, was not essential in splitting the complement, but was merely an associated phenomenon. The correctness of the above deduction will be established by the experiments recorded in the following pages.

The so-called splitting of complement may be effected by certain procedures without causing the separation of the globulin fraction of complement. In one of these procedures, distilled water in certain proportions is used, and in another serum is treated with phosphates. The inactivation of complement by distilled water is doubtless due to the action upon the complement of dissociated ions of electrolytes contained in the serum, and the inactivation by a mix-

* Received for publication, April 1, 1912.

ture of acid and alkaline phosphates (the acid being in excess) seems similar to the action of any other acid, but the presence of an alkaline phosphate modifies the reaction of the medium in such a manner that the globulin is kept in solution.

Although the phenomena of splitting complement as brought about by these chemical or physical interferences appear to be similar in mechanism to those effected by complement fixation, the complex biological reactions of the latter are difficult to analyze, and will be left for future study.

Under separate headings, we shall give the results of the other ways of splitting complement.

COMPLEMENT-SPLITTING WITH WATER.

Complement, as long as it is handled with an isotonic solution of sodium chlorid, retains its activity on dilution. On the other hand, complement rapidly loses its activity if it is diluted to a certain extent with an isotonic sugar (saccharose) solution, as was first pointed out by Sachs and Teruuchi. Thus these authors found that complement becomes completely inactive within one hour when diluted ten times with an isotonic sugar solution. The inactivation was considered by them to be due to a ferment which in a sodium chlorid-free medium manifests its activity upon complement.[1]

The above phenomenon appears, however, to be caused by some other factors than a ferment. In the case of inactivation by means of distilled water we must consider first what occurs if a solution containing two different salts of varying dissociabilities are diluted ten or more times with water. In the original concentration, the stronger electrolyte hinders the dissociation of the weaker, but when sufficiently diluted the dominating salt permits the weaker electrolyte to dissociate more fully. Sodium chlorid is a much stronger electrolyte than $NaHCO_3$ or Na_2CO_3 and it doubtless interferes with the free dissociation of the latter salts in the concentration present in serum; but when diluted ten times, its concentration no longer hinders the free dissociation of the carbonates. Thus, it becomes evident that the complement which may remain unaffected

[1] Bronfenbrenner, J., and Noguchi, H., *Jour. Exper. Med.*, 1912, xv, 598 (tables XIV and XV, A).

by the carbonates in the presence of sodium chlorid in the original concentration will be exposed to the action of the latter as soon as the sodium chlorid concentration is much less, and this is exactly what happens when serum is diluted with water. This, of course, will not occur as long as a sufficient amount of the chlorid is contained in the diluent (table I). That the change in the tonicity of the mixture (serum and diluent) has nothing to do with the loss of complement activity is shown by the fact that the use of an isotonic solution of a non-electrolyte, such as cane sugar, does not prevent the inactivation of complement.

We shall next see if the inactivation of complement by dilution with water or cane sugar solution is really an alkali inactivation. That this is the case is shown by the fact that an acid and also a few amphoteric substances may reactivate the inactive complement.[2]

TABLE I.

The Inactivation of Complement in a Salt-Free Isotonic Medium.

	Complement diluted to 40 % in 0.9 % NaCl and incubated at 37° C. for 1 hr.	Complement diluted to 40 % with 7.2 % sugar and incubated at 37° C. for 1 hr.
	0.2 c.c. of this dilution is used with varying amounts of 7.2 % sugar.	0.2 c.c. of this dilution is used with varying amounts of 0.9 % NaCl.
	Total volume made equal to 1 c.c. with 0.9 % NaCl.	Total volume made equal to 1 c.c. with 7.2 % sugar.
Varying amounts of sugar or NaCl added. 0.1	C.H.	No H.
0.15	C.H.	Sl.H.
0.2	C.H.	C.H.
0.3	C.H.	C.H.
0.4	C.H.	C.H.
0.5	C.H.	C.H.
0.7	C.H.	C.H.
1 c.c.	C.H.	C.H.

COMPLEMENT-SPLITTING BY MEANS OF PHOSPHATES.

Michaelis and Skwirsky described a method by which complement can be split into end- and mid-pieces. In this method one cubic centimeter of complement (1:10) is mixed with two cubic centimeters of mixture A-B, which consists of sixteen parts of M/7 NaH_2PO_4 (solution A) and one part of M/7 Na_2HPO_4 (solution B), and to this are added one cubic centimeter of amboceptor (fifty

[2] Bronfenbrenner, J., and Noguchi, H., *loc. cit.*, tables XIV and XV.

TABLE II.

The Effect of Hydrochloric Acid upon the Inactivation of Complement by Distilled Water.

		0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.04 c.c.	0.03 c.c.	0.02 c.c.
Native complement + 0.9% NaCl added after 15 min.	1.0 c.c.											
	9.0 c.c.								C.H.	F.C.H.	Sl.H.	No H.
Complement + distilled water + 10% NaCl added after 15 min.	1.0 c.c.											
	8.2 c.c.											
	0.8 c.c.						C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
Complement + distilled water + 10% NaCl added after 30 min.	1.0 c.c.											
	8.2 c.c.											
	0.8 c.c.	No H.										
Complement HCl N/250 + NaOH N/25 in 10% NaCl added after 15 min.	1.0 c.c.											
	8.2 c.c.											
	0.8 c.c.		C.H.	F.C.H.	Sl.H.	Sl.H.	No H.					
Complement HCl N/250 + NaOH N/25 in 10% NaCl added after 30 min.	1.0 c.c.											
	8.2 c.c.											
	0.8 c.c.		C.H.	F.C.H.	Sl.H.	Sl.H.	No H.					
Complement HCl N/250 + NaOH N/25 in 10% NaCl added after 2 hrs.	1.0 c.c.											
	8.2 c.c.											
	0.8 c.c.		C.H.	F.C.H.	Sl.H.	Tr.H.	No H.					
Complement + 0.9% NaCl after 2 hrs. (control).	1.0 c.c.											
	9.0 c.c.								C.H.	Mch.H.		

units) and one cubic centimeter of 5 per cent. sheep corpuscles. After incubation for one hour at 37° C. the mixture is centrifugalized and the supernatant fluid is separated from the deposited corpuscles. To the supernatant fluid, two cubic centimeters of solution B are added and the whole is called the end-piece. The corpuscles are suspended in salt solution and are used as persensitized corpuscles (sensitized and carrying the mid-piece of complement). When the persensitized corpuscles are mixed with the end-piece they undergo hemolysis, while the sensitized corpuscles are not acted upon by the end-piece.

The above phenomenon is probably explained by the fact that complement is highly sensitive to the action of various acids, alkalis, and salts, and undergoes a reversible inactivation. It is not at all improbable, therefore, that the mixture of solutions A and B, which is strongly acid to phenolphthalein, renders the complement inactive, while the persensitization goes on without hinderance in the same medium. The acidity of the supernatant fluid is, in this instance, to a certain degree reduced by the later addition of solution B so that the inactivated complement, which is near the border of rever-

TABLE III.

Reaction and Anticomplementary Activity of Different Mixtures of Phosphates.

	Reaction of 1 c.c.		Anticomplementary activity upon 0.04 c.c. of complement + 2 units of amboceptor.	
	Indigo-alizarin.	Phenolphthalein.	Complete hemolytic dose.	Non-hemolytic dose.
Solution A. 27.4 gm. NaH_2PO_4 per 1,000 c.c.	Acid +0.4 c.c. NaOH N/100	Acid 24 c.c. NaOH N/100	0.07 c.c.	0.2 c.c.
Solution B. 51.4 gm. Na_2HPO_4 per 1,000 c.c.	Alkali 15 c.c. HCl N/100	Alkali 0.7 c.c. HCl N/100	0.5 c.c.	1 c.c.
Mixture A–B. 16 c.c. A+1 c.c. B.	Alkali 0.45 c.c. HCl N/100	Acid 22 c.c. NaOH N/100	0.1 c.c.	0.25 c.c.
Neutralized Mixture. 1 c.c. A–B+1 c.c. B.			0.5 c.c.	1 c.c.

sion, regains its activity whenever an adequate quantity of certain alkali (table IV) or amphoteric compounds is introduced (table V).

TABLE IV.

Reactivation of Complement Inactivated by Phosphates.

	NaOH N/1 in c.c.									
	0.1	0.07	0.05	0.03	0.02	0.01	0.005	0.001	0.0007	0.0005
Complement[3] 0.04 c.c. + 27.4/1,000 NaH_2PO_4 0.2 c.c. in each tube + 3 units of amboceptor.	Alkali hemolysis.			C.H.	C.H.	C.H.	C.H.	No H.	No H.	No H.
		Zone neutral to phenolphthalein.						Zone neutral to alizarin-indigo-carmin.		

	HCl N/1 in c.c.									
	0.2	0.1	0.07	0.05	0.03	0.0275	0.025	0.02	0.01	0.007	0.005
Complement[3] 0.04 c.c. + 51.4/1,000 Na_2HPO_4 1 c.c. in each tube + 3 units of amboceptor.	Acid hemolysis.										
			No H.	Sl.H.	C.H.	Sl.H.	No H.	No H.	No H.	No H.	No H.
	Zone neutral to phenolphthalein.								Zone neutral to alizarin-indigo-carmin.		

From the foregoing experiments it will be seen that the inactivated complement is to be found in the supernatant fluid in a reversible state. The reversion is accomplished by relieving the complement of the acid reaction by any suitable substance. The reason that the corpuscles digested in the Michaelis-Skwirsky mixture are capable of reversing the inactivated complement may be due to the absorption of a certain amount of serum constituents (probably the globulin fraction), also the absorption of the alkaline phosphate by certain constituents of the cells. It appears from the following experiments that the presence of amboceptor has a certain influence in enabling the corpuscles to absorb them, because without the amboceptor the persensitization is seen to be less complete (table VI).

That the end-piece of complement as obtained by the method of Michaelis and Skwirsky is in a general way similar to those obtained by other processes is well shown in the following experiments. It is most striking that alanin plays beautifully the rôle of the mid-piece with all end-pieces here studied (table VII, C).

[3] The complement was incubated with the phosphates at 37° C. for 30 minutes, then alkali and acid, respectively, were added and the whole was reincubated for 30 minutes.

TABLE V.

Reactivation of Complement Inactivated by Phosphates.

Complement 0.04 c.c. + Na_2HPO_4 (solution 51.4gm./1,000) 1 c.c. in each tube incubated for 30 minutes at 37° C. Then varying amounts of reactivating substances were added, the volume brought to 2 c.c. in each tube and incubated for 30 minutes at 37° C.; finally 10 units of amboceptor and 0.1 c.c. of a 10% suspension of corpuscles were added and again incubated for 30 minutes.

	0.05 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.4 c.c.	0.5 c.c.	0.6 c.c.	0.7 c.c.	1 c.c.	Control.	Na_2HPO_4. (No complement.) 0.5 c.c.	1 c.c.
Egg-white (1:5)					No H.	Sl.H.	F.C.H.	C.H.	C.H.			No H.
Serum 56° C. (sheep) ...			No H.	Sl.H.	Mch.H.	C.H.						No H.
Alanin 3% in NaCl (0.9%)	No H.	Sl.H.	F.C.H.	C.H.								No H.
HCl N/10 in 0.9% NaCl.	No H.	F.C.H.	C.H.	C.H.	Sl.H.	No H.					No H.	C.H.
0 Noreactivating substances										No H.		

Same as above except that 0.2 c.c. NaH_2PO_4 (solution 27.4/1,000) was used instead of 1 c.c. of Na_2HPO_4.

	0.05 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.4 c.c.	0.5 c.c.	0.6 c.c.	0.7 c.c.	1 c.c.	Control.	NaH_2PO_4. (No complement.) 0.3	0.5
Egg-white (1:5)				No H.	Sl. H.	F.C.H.	Sl.H.	No H.				
Serum 56° C. (sheep) ...	Tr.H.	Sl.H.	C.H.	C.H.	C.H.	F.C.H.	No H.					
Alanin 3% in 0.9% NaCl						No H.	No H.	Sl.H.	F.C.H.			
NaOH N/10 in 0.9% NaCl	F.C.H.	C.H.	C.H.	C.H.	F.C.H.	Sl.H.					No H.	C.H.
0 Noreactivating substances										No H.		

TABLE VI.

Can Corpuscles be Persensitized without Amboceptor, or Complement, or Both?

Tubes.	1	2	3	4	5	6
	Complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. Corpuscles (5%) 0.2 c.c.	Heated complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. Corpuscles (5%) 0.2 c.c.	0.9% NaCl 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. Corpuscles (5%) 0.2 c.c.	Complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. NaCl 0.9% 0.2 c.c. Corpuscles (5%) 0.2 c.c.	Complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. NaCl (0.9%) 0.2 c.c.	Complement (1:10) 0.2 c.c. Mixture A-B 0 Amboceptor 0 Corpuscles (5%) 0.2 c.c. NaCl (0.9%) 0.6 c.c.
Remarks		Heated complement	No complement	No amboceptor	No corpuscles	No mixture A-B No amboceptor
All mixtures incubated for 60 minutes at 37° C. and corpuscles separated by centrifugalization (to be used later).						
0.4 c.c. of solution B (Na_2HPO_4) added to all the supernatant fluids except the last (6).						0.4 c.c. NaCl added.
To the different amounts of the supernatant fluid of each tube the corpuscles of each of the other tubes were added and again incubated; results taken after 1 hour.						

(Continued on page 633.)

TABLE VI (*Continued*).

Corpuscles from tubes.	Supernatant fluid from tube 1.				Supernatant fluid from tube 2.			Supernatant fluid from tube 3.		
	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	1 c.c.	0.7 c.c.	0.5 c.c.	1 c.c.	0.7 c.c.	0.5 c.c.
1	C.H.	F.C.H.	Sl.H.	No H.	No H.			No H.		
2	C.H.	F.C.H.	Tr.H.	No H.	No H.			No H.		
3	C.H.	Mch.H.	No H.		No H.			No H.?		
4	F.C.H.	Sl.H.	No H.		No H.			No H.		
6	F.C.H.	Sl.H.	No H.		No H.			No H.		
Non-treated corpuscles (5%) 0.2 c.c.	F.C.H.	Sl.H.	No H.		No H.			No H.		

Corpuscles from tubes.	Supernatant fluid from tube 4.				Supernatant fluid from tube 5.				Supernatant fluid from tube 6.		
	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.
1	C.H.	F.C.H.	Tr.H.	No H.	C.H.	Sl.H.	No H.		C.H.	Mch.H.	No H.
2	C.H.	Mch.H.	No H.		C.H.	Mch.H.	No H.		C.H.	Mch.H.	No H.
3	C.H.	Mch.H.	Tr.H.	No H.	C.H.	Mch.H.	No H.		C.H.	Sl.H.	No H.
4	C.H.	Sl.H.	No H.		C.H.?	No H.	No H.		C.H.	Mch.H.	No H.
6	C.H.	Mch.H.	Tr.H.?	No H.	F.C.H.	Mch.H.	No H.		C.H.	Sl.H.	No H.
Non-treated corpuscles (5%) 0.2 c.c.	C.H.	Sl.H.	No H.		F.C.H.	Mch.H.	No H.		C.H.	Mch.H.	No H.
	Amboceptor (20 units) 0.2 c.c. added.								Amboceptor (20 units) 0.2 c.c. added.		

TABLE VII.

Activity of Different End-Pieces with Various Mid-Pieces and Alanin.

	0.5 c.c. of 5 % suspension of fresh corpuscles + 50 units of amboceptor.		Same amount of persensitized corpuscles.		Same amount of sensitized corpuscles.		0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor + 0.5 c.c. of mid-piece (CO_2).		0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor + 0.5 c.c. HCl mid-piece.		0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor + 0.3 c.c. of 3 % alanin.	
Amounts of end-piece used.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.
(A) End-piece (CO_2) made isotonic.	No H.	No H.	C.H.	C.H.	F.C.H.	C.H.	Mch.H.	No H.	C.H.	C.H.	C.H.	C.H.
(B) End-piece (HCl) made neutral and isotonic.	No H.	No H.	C.H.	C.H.	Sl.H.(?)	No H.	F.C.H.	Sl.H.	C.H.	C.H.	Sl.H.	No H.
Amounts of Skwirsky's[1] end-piece used.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.
(C) Skwirsky's end-piece neutralized by solution B.	No H.	No H.	C.H.	C.H.	Sl.H.(?)	No H.	C.H.	Sl.H.	C.H.	F.C.H.	C.H.	Sl.H.

[1] Skwirsky's end-piece being more diluted, it was necessary to increase the quantity accordingly.

COMPLEMENT-SPLITTING IN COMPLEMENT DEVIATION PHENOMENA.

Much more complicated is the phenomenon first described by Skwirsky, who found that the supernatant fluid of the mixture containing a positive syphilitic serum, syphilitic antigen, complement, sheep's corpuscles, and antisheep amboceptor, has the power to cause hemolysis upon a fresh lot of sheep corpuscles when the latter are previously persensitized. By the term of persensitization is meant the digestion of washed sheep corpuscles in a mixture of sixteen

TABLE VIII.

Action of Mixture A-B upon the Substances Concerned in the Wassermann Reaction.

1	Syphilitic serum (heated at 56° C.) 0.1 c.c. Antigen (0.3 %) (lipoids) 0.5 c.c. Complement (1:10) 0.5 c.c. NaCl (0.9%) 0.4 c.c. 1.5 c.c.	Each of the tubes contained this mixture (1.5 c.c.) The tubes were incubated at 37° C. for 30 min. then varying amounts of A–B mixture were added and the volume was brought up to 2.5 c.c. with NaCl (0.9 %). After the second incubation for 30 min. at 37° C., 0.5 c.c. of 5 % sheep corpuscles and 50 units of amboceptor were added and the whole was again incubated for 30 min. at 37° C.							
	Varying amounts of the mixture A–B.	1 c.c.	0.7c.c.	0.5c.c.	0.3c.c.	0.2c.c.	0.1c.c.	0.07c.c.	0.05c.c.	0
	Result of the hemolytic test.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
2	In this series, corpuscles were digested with amboceptor and mixture A–B for 30 min. at 37° C., and then to each tube 1.5 c.c. of the above mixture of complement, antigen, syphilitic serum, and NaCl were added. The amounts of substances used were the same as above.									
	Varying amounts of mixture A–B + corpuscles 5 % 0.5 c.c. + amboceptor 50 units.	1 c.c.	0.7 c.c.	0.5 c.c.	0.3c.c.	0.2 c.c.	0.1 c.c.	0.07c.c.	0.05c.c.	0
	Results of the hemolytic test after additions of 1.5 c.c. of the above mixture (1).	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	No H.
3	Corpuscles (0.5 c.c. of 5 % suspension) were digested for 30 min. at 37° C. with complement (1:10) 0.5 c.c. + amboceptor (50 units) 0.5 c.c. + mixture A–B 1 c.c.; then the corpuscles were separated by centrifugalization and acted upon by varying amounts of mixture 1.									
	Amounts of mixture 1 added; total volume made equal to 3.5 c.c.	1 c.c.	0.7c.c.	0.5c.c.	0.3c.c.	0.2 c.c.	0.1 c.c.	0		
	Results of the hemolytic experiment.	C. H.	C. H.	C. H.	Sl. H.	No H. (?)	No H.	No H.		

parts of M/7 solution of NaH_2PO_4 and one part of M/7 solution of Na_2HPO_4, to which a definite amount of complement and a large quantity of antisheep amboceptor have also been added (table IX, 2). The corpuscles digested in this mixture are hemolyzable by the supernatant fluid separated from a positive fixation test. The ordinary corpuscles with or without amboceptor are not hemolyzed by the supernatant fluid. Thus, this phenomenon was explained by them as being due to the presence of the end-piece of the complement in the supernatant fluid which, by virtue of the absorption of the mid-piece of complement by the sensitized corpuscles in the digestion procedure, becomes active upon the latter.

This phenomenon was confirmed by various investigators and we have also obtained a similar result (table IX).

Curiously enough, however, this apparent splitting of complement during the fixation by the Wassermann or Bordet-Gengou reaction does not take place when the antihuman hemolytic system is used (table X).

It is also interesting to note that the supernatant fluid of the Wassermann reaction which is quite active for the persensitized sheep corpuscles is almost inactive upon fresh sheep corpuscles when varying amounts of the mixture of solution A-B and fifty units of amboceptor are added (table XI, B). Apparently the addition of the A-B mixture and amboceptor alone is not sufficient to make the Wassermann supernatant fluid active. On the other hand, when the mixture A-B is allowed to act previously upon the sheep corpuscles with fifty units of antisheep amboceptor, the Wassermann supernatant fluid shows a certain amount of hemolytic effect. Here it seems as if the A-B mixture had so altered the corpuscles that they became to a certain extent hemolyzable by the Wassermann supernatant fluid (table XI).

It was also found that the persensitization of sheep corpuscles takes place in a hypertonic sodium chlorid or sugar solution, as indicated in the following experiments (table XII). At the same time it is interesting to notice that the Wassermann supernatant fluid is inactive upon the corpuscles persensitized when the medium is hypertonic.

The heating of the Wassermann supernatant fluid to 56° C. for

TABLE IX.

Splitting of Complement in the Wassermann Reaction with the Antisheep Hemolytic System.

1	2	3
Designated as Wassermann supernatant fluid.	Designated as persensitized corpuscles.	Designated as sensitized corpuscles.
Syphilitic serum (56° C.) 0.1 c.c. Antigen (lipoids) (0.3 %) 0.5 c.c. Complement (1:15) 0.5 c.c. NaCl (0.9 %) 0.4 c.c.	Mixture A–B 1 c.c. Corpuscles (5 %) 0.5 c.c. Amboceptor (50 units) 0.5 c.c. Complement (1:10) 0.5 c.c.	Suspension of corpuscles (10 %) 2.5 c.c. Amboceptor (50 units) 2.5 c.c.
Incubated 30 min. at 37° C.	Incubated 60 min. at 37° C. Then corpuscles were separated by centrifugalization and resuspended in 0.5 c.c. of 0.9 % NaCl.	Incubated 60 min. at 37° C. Then corpuscles were separated by centrifugalization and resuspended in 0.5 c.c. of 0.9 % NaCl.

Wassermann supernatant fluid (0.5 c.c. of complement 1:15 diluted in 1.5 c.c. of fluid).

Amounts of Wassermann supernatant fluid used.	+ 0.5 c.c. of persensitized corpuscles.	+ 0.5 c.c. of sensitized corpuscles: + 0	+ Mid-piece[6] (CO_2) 1:1	+ Alanin 3 % 0.3 c.c.
1.0 c.c.	C.H.	No H.	No H.	No H.
0.7 c.c.	C.H.			
0.5 c.c.	Sl.H.			
0.3 c.c.	No H.			
0.2 c.c.				

Complement (1:15) 0.5 c.c. + NaCl (0.9 %) 1 c.c. (0.5 c.c. of complement 1:15 diluted in 1.5 c.c. of salt solution).

Amounts of dilution of complement used.	+0.5 c.c. of persensitized corpuscles.	+ 0.5 c.c. of sensitized corpuscles: + 0	+ Mid-piece[5] (CO_2) 1:1	+ Alanin 3 % 0.3 c.c.
1.0 c.c.	C.H.	C.H.		
0.7 c.c.	C.H.	C.H.		
0.5 c.c.	Sl.H.	C.H.		
0.3 c.c.	No H.	F.C.H.	C.H.	C.H.
0.2 c.c.		Sl.H.	Mch.H.	Sl.H.
0.15 c.c.		No H.	No H.	No H.

[6] The amount of mid-piece used was equal to the amount of complement present in the corresponding amount of dilution used; namely, 1 c.c. of complement was broken up by CO_2 and the globulin fraction, collected by centrifugalization, dissolved in 15 c.c. of NaCl and 0.3 c.c., 0.2 c.c., 0.15 c.c., 0.07 c.c., respectively, were taken for 1 c.c., 0.7 c.c., 0.5 c.c., 0.3 c.c., of the Wassermann supernatant fluid.

TABLE X.

The Presence of End-Piece in the Supernatant Fluid of the Wassermann and Bordet-Gengou Reactions Tested with the Antihuman Hemolytic System.[6]

Designated as Wassermann supernatant fluid (comparable to table IX, 1).

Syphilitic serum (56° C.)	0.1 c.c.
Antigen (0.3 %)	0.5 c.c.
Complement (1:15)	0.5 c.c.
NaCl (0.9 %)	0.4 c.c.

Incubated 30 min. at 37° C.

	Mid-piece (CO_2).		No mid piece (control).
	0.04 c.c.	0.02 c.c.	
The above Wassermann[7] supernatant fluid 1.5 c.c.	No H.	No H.	No H.
End-piece (CO_2) 0.04 c.c.	C.H.	C.H.	No H.
0 (control).	No H.		

+ 0.5 c.c. of persensitized corpuscles.

Wassermann supernatant fluid 1.5 c.c.	No H.

Designated as mixture II.

Egg-white (1:10)	1 c.c.
Anti-egg serum (1:10)	1.5 c.c.
NaCl (0.9 %)	7.5 c.c.

Incubated 60 min. at 37° C., then shaken up and varying amounts incubated again with 0.1 c.c. of 40 % complement.

	Amount of mixture II added to 0.1 c.c. of 40 % complement.				
	0	0.2 c.c.	0.4 c.c.	0.7 c.c.	1 c.c.
0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor	C.H.	C.H.	C.H.	Sl. H.	No H.
0.5 c.c. of persensitized corpuscles.	C.H.	F.C.H.	Sl. H.	No H.	

Designated as supernatant fluid III.

Sheep serum (56° C.)	0.1 c.c.
Antisheep serum (56° C.)	0.1 c.c.
Complement (40 %)	0.1 c.c.
NaCl (0.9 %)	0.7 c.c.

Incubated 60 min. at 37° C., centrifugalized and supernatant fluid examined.

	+ 0.2 c.c. mid piece.	No mid-piece.
Supernatant fluid III 1 c.c.	No H.	No H.
End-piece (CO_2) 0.04 c.c.	C.H.	No H.

+ 0.5 c.c. of persensitized corpuscles.

Supernatant fluid 1 c.c.	No H.
Native complement 0.04 c.c.	C.H.
End-piece (CO_2) 0.04 c.c.	C.H.

[6] The table is arranged to show the three different procedures used in each of these experiments, all of which show the same result, contrary to the experiments with antisheep hemolytic system.

[7] In this experiment the human hemolytic system was used and in all the tubes the total volume was brought to 2 c.c. and when necessary 50 units of amboceptor and 0.5 c.c. of a 5 % suspension of human corpuscles were added.

TABLE XI.

The Effect of Mixture A-B upon Sheep Corpuscles and Wassermann Supernatant Fluid, Respectively.

	Amounts of solution A-B added.							0.5 c.c. of persensitized corpuscles.	0.5 c.c. of sensitized corpuscles.
								Controls.	Controls.
A. Wassermann[8] supernatant fluid 1.5 c.c. added to all. After additions of A–B, incubated at 37° C. for 30 min. 0.5 c.c. of 5% sheep corpuscles and 50 units amboceptor. Incubated for 60 min., and results taken.	1 c.c.	0.7 c.c.	0.5 c.c.	0.1 c.c.	0.5 c.c.	0.03 c.c.	0.01 c.c.	0	0
	No H.	No H.	No H.	No H.	No H.	No H.	No H.	C.H.	No H.
	Amounts of solution A-B added.							Controls	Controls
B. Corpuscles (5%) 0.5 c.c. Amboceptor (50 units) 0.5 c.c. Varying amounts of solution A-B. Incubated at 37° C., for 30 min., then[8] 1.5 c.c. Wassermann supernatant fluid added to each tube and after incubation for 60 min., results taken.	1 c.c.	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.	0.03 c.c.	0	0
	No H.(?)	Sl.H.(?)	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	No H.	C.H.	No H.

[8] See table IX, mixture I.

TABLE XII.

Does Persensitization Occur in a Hypertonic Medium?

Complement (1:15) 0.5 c.c. Amboceptor 50 units 0.5 c.c. Mixture A-B 1.0 c.c. Sheep corpuscles 5% 0.5 c.c. 0.9 % NaCl solution 0.5 c.c.				Complement (1:15) 0.5 c.c. Amboceptor 50 units 0.5 c.c. Mixture A-B 1.0 c.c. Sheep corpuscles 5% 0.5 c.c. 10% NaCl solution 0.5 c.c.		Comp ement (1:15) 0.5 c.c. Amboceptor (50 units) 0.8 c.c. Mixture A-B 1.0 c.c. Sheep corpuscles (5%) 0.5 c.c. 10 times isotonic (72%) sugar solution 0.5 c.c.	
After 30 min. incubation at 37° C., corpuscles separated and used for the following experiment.				After 30 min. incubation at 37° C., corpuscles separated and used for the following experiment.		After 30 min. incubation at 37° C., corpuscles separated and used for the following experiment.	
1.5 c.c. Wassermann supernatant fluid			1.5 c.c. of 0.9% NaCl +0.5 c.c. of 0.9% NaCl	1.5 c.c. of 0.9% NaCl +0.5 c.c. of 0.9% NaCl	1.5 c.c. of Wassermann supernatant fluid +0.5 c.c. of 0.9% NaCl	1.5 c.c. of 0.9% NaCl +0.5 c.c. of 0.9% NaCl	1.5 c.c. of Wassermann supernatant fluid +0.5 c.c. of 0.9% NaCl
+0	+0.5 c.c. of 10% NaCl	+0.5 c.c. of 72% Sugar					
C.H.	No H.	F.C.H.	No H.	No H.	C.H.	No H.	C.H.

TABLE XIII.

The Activation of Different End-Pieces by Different Mid-Pieces and Alanin in the Presence of Sheep or Human Corpuscles.

		End-piece (CO_2) isotonic, 0.4 c.c.		End-piece (HCl) neutralized and isotonic, 0.4 c.c.		Wassermann supernatant fluid, 1.5 c.c.		End-piece (Skwirsky)[9] neutralized by B, 3.5 c.c.		0.9% NaCl, 3.5 c.c.	
		Corpuscles.		Corpuscles.		Corpuscles.		Corpuscles.		Corpuscles.	
		Human.	Sheep.	Human.	Sheep.	Human.	Sheep.	Human.	Sheep.	Human.	Sheep.
Mid-piece (CO_2) 0.2 c.c.	50 units of amboceptor and 0.5 c.c. of 5% puscles	Sl.H.	Mch.H.	Sl.H.	C.H.	No H.	No H.	No H.	F.C.H.	No H.	No H.
Mid-piece (HCl) 0.2 c.c.		Mch.H.	F.C.H.	Mch.H.	C.H.	No H.	No H.	No H.	F.C.H.	No H.	No H.
Alanin (3%) 0.3 c.c.		Mch.H.	Mch.H.	C.H.	C.H.	No H.	No H.	No H.	No H.	No H.	No H.
Mid-piece of heated complement (CO_2) 0.2 c.c.		Mch.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	Sl.H.	No H.	No H.
0.5 c.c. of persensitized corpuscles.		Sl.H.	C.H.	Sl.H.	C.H.	No H.	F.C.H.	F.C.H.	C.H.	No H.	No H.

[9] Skwirsky's end-piece is the supernatant fluid (described in table IX, 2) resulting after persensitized corpuscles are removed from the mixture of A-B + complement + amboceptor + corpuscles. This supernatant fluid (2.5 c.c.) is to be neutralized by 1 c.c. of solution B and the result is designated Skwirsky's end-piece.

thirty minutes destroys its activity upon persensitized sheep corpuscles. The supernatant fluid from the positive Wassermann serum in which an inactivated complement, instead of fresh complement, had been used, is totally inactive upon the persensitized corpuscles, while the persensitization of the corpuscles can be accomplished by means of an inactivated as well as an active complement (table VI, 2).

From the fact that the sheep corpuscles separated from a positive Wassermann or Bordet-Gengou fixation test remain sensitized, but not persensitized, it is evident that the mid-piece in this instance interacts with the mixture of syphilitic serum and lipoids or antibody and antigen, thus becoming incapable of persensitizing the sensitized corpuscles. Just what kind of interaction has taken place is at present undetermined, but one point is certain and that is that the mid-piece is completely used up.

In this respect we may cite here an interesting phenomenon already observed by Noguchi and Bronfenbrenner (1910): namely, the interference exerted by certain amphoteric substances (egg-white, inactivated sera, etc.) upon the fixation phenomenon. Apparently the fixing molecules of the antibody-antigen combination are saturated by these substances which in this respect resemble the mid-piece.

GENERAL CONSIDERATION.

Thus far, most investigators have made but little distinction between the splitting phenomenon obtained by chemical interference and that which takes place in the biological phenomenon known as complement fixation. In this study we have shown that these two sets of phenomena have certain fundamental differences and that the so-called complement-splitting by physical conditions leading to chemical interaction, or directly by chemical means, is not a real splitting of the complement, but an inactivation of the active principle of complement through an alteration in the reaction of the medium caused by an excess of either anions or kations. The modification of the reaction of the medium may cause a more or less definite combination of the complement with the free ions, but the latter can readily be removed by an appropriate number of opposite

ions, and render the complement active once more. The fluids that have hitherto been regarded as containing the end-piece of complement, as a matter of fact, contain the whole complement temporarily deprived of its activity by certain ions derived either from the salt constituents of the serum itself under a modified physical condition (dialysis against water or dilution with water) or introduced in the form of dissociable electrolytes.

On the other hand, the splitting of complement in the fixation reaction seems far more complicated than that caused by the physical or chemical procedures. The supernatant fluid from the fixation test differs from all the other end-pieces prepared by chemical methods in being active upon persensitized sheep corpuscles only (not upon human corpuscles). The addition of various mid-pieces obtained by different methods to sensitized sheep corpuscles does not render the Wassermann supernatant fluid active (table XIII). It is quite remarkable that the persensitized sheep corpuscles are, on the other hand, easily attacked, not only by the supernatant fluids of fixation tests, but also equally well by the other end-pieces. It is not at all improbable that in the fixation reaction, where so many factors come into play, there is a most complicated physical as well as chemical interaction leading to such an entangled mixture of factors that a substance carrying one set of ions alone cannot reverse the activity of complement, and hence the reversion takes place only when certain electrolytes with both ions are employed. At all events there seems to be no doubt that the inactivation of complement is far more complicated in the Wassermann reaction or the Bordet-Gengou phenomenon than in the inactivation by physical or chemical means. Nevertheless, no one has as yet proved conclusively that the supernatant fluid of a fixation test necessarily contains the end-piece of complement.

A QUANTITATIVE STUDY OF THE EFFECTS OF ADRENALIN ON THE PUPILS OF RABBITS AFTER REMOVAL OF A SUPERIOR CERVICAL GANGLION

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[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XV, No. 1, 1912.]

A QUANTITATIVE STUDY OF THE EFFECTS OF ADRENALIN ON THE PUPILS OF RABBITS AFTER REMOVAL OF A SUPERIOR CERVICAL GANGLION.*

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Among the most characteristic effects of adrenin,[1] or epinephrin,[1] upon the animal body is its dilating action upon the pupil of mammals. It was first discovered by Lewandowsky[2] and confirmed by Boruttau[3] and Langley.[4] In their experiments, however, the dilatation produced was very transient, reaching its maximum within ten to thirty seconds after the intravenous injection and soon disappearing completely, the entire effect lasting two to four minutes at most. Subcutaneous injections or instillations into the conjunctival sac produced no effect, and the assumption was made that an early oxidation of the suprarenal extract within the tissues prevented its entrance into the blood-stream in the active form.

It was discovered later, however, by Meltzer and C. M. Auer[5] that the removal of a superior cervical ganglion favors greatly the dilating action of adrenin upon the pupil of the corresponding side, so that not only an intravenous, but even a subcutaneous injection, or an instillation in the corresponding conjunctival sac, caused a prompt dilatation of the corresponding pupil. Furthermore, with a sufficiently large dose of the adrenin, the dilatation of the pupil frequently lasted several hours. This is the more striking since it is in contrast to the fleeting effect which the administration of the

* Received for publication, April 3, 1912.

[1] Designations for the active principle of adrenal extracts, in contradistinction to the terms employed for definite commercial preparations.

[2] Lewandowsky, *Arch. f. Physiol.*, 1899, 360.

[3] Boruttau, *Arch. f. d. ges. Physiol.*, 1899, lxxviii, 112.

[4] Langley, *Jour. Physiol.*, 1901–2, xxvii, 237.

[5] Meltzer and (C. M.) Auer, *Am. Jour. Physiol.*, 1904, xi, 28.

extract produced upon the pupil on the side with the intact ganglion. To obtain such striking effects the administrations of the adrenin had to be made some time after the removal of the ganglion, the necessary interval for rabbits being about twenty-four hours, and for cats about forty-eight hours.

The doses of adrenin employed in the experiments of Meltzer and Auer were calculated to bring out a maximum effect and therefore were quite large in most cases. In recent experiments upon the relations of the splanchnic nerves to the secretion of the adrenals[6] it became necessary to have some knowledge of the degree and duration of the effect which small doses of adrenin may exert upon the pupils. I have therefore carried out a series of experiments in which the dilating effect of adrenin upon the pupil was studied essentially from a quantitative point of view. This was done by the use of graded doses, and the attempt was made to find the smallest dose to which the deganglionized pupil will respond with a recognizable reaction, as well as to establish, if possible, the amount and duration of the dilatation produced by various larger doses. In addition, a few experiments were made to establish more exactly, how soon after the removal of the ganglion the increased sensitiveness of the pupil develops.

METHODS.

The experiments were made on rabbits. White ones were used exclusively, because of the ease with which the border of their pupils can be seen. From such rabbits, one superior cervical sympathetic ganglion was removed under morphin. The right and left ganglia were removed in about an equal number of animals. In the majority of the experiments the injections were made only after twenty-four hours, but in a few instances they were given during the first twenty-four hours in order to see how soon the increased sensitiveness appears. Only intravenous injections were used. In making the injections of adrenalin into a marginal ear vein the animals were held firmly in the hands of an assistant or were gently stretched out on a Cannon board. No anesthetic was required.

[6] Joseph and Meltzer, *Proc. Am. Physiol. Soc., Am. Jour. Physiol.*, 1912, xxix, p. xxxiv.

The solution used was the 1 : 1,000 adrenalin chlorid solution (Parke, Davis and Company). In nearly all cases it was used in this strength. In a few instances in which very small doses were given it was necessary, for accuracy, to dilute this solution to one half. The doses of adrenalin used, stated in terms of cubic centimeters of the 1 : 1,000 solution per kilo of body weight, were: $\frac{1}{50}$, $\frac{1}{30}$, $\frac{1}{20}$, $\frac{1}{10}$, and $\frac{2}{10}$ of a cubic centimeter. A tuberculin (Record) syringe, graduated to fiftieths of a cubic centimeter, was used in making the injections.

After the injection the animal was released at once and the pupil readings were begun within a minute and repeated every few minutes while the dilatation lasted. The diameter of the pupil was estimated by comparing it with a pupillometer, consisting of a series of black discs of known diameter painted on a white background. The pupil was rarely round, and in the protocols, where two diameters are given for a single pupil, the first one is the horizontal as the animal stands erect, and the second, the vertical diameter. The latter is almost always the greater. The light conditions in individual experiments were as nearly constant as possible.

EXPERIMENTAL RESULTS.

Adrenalin, $\frac{1}{50}$ of a Cubic Centimeter per Kilo of Body Weight.— In this series there were eight experiments (table I). In six of them the ganglion had been removed more than twenty-four hours previously. The dilatation produced was, with one exception, definite in all cases. It varied for the six animals between 0.5 of a millimeter (which is too small an amount to be considered a definite dilatation) and 3 millimeters, the average being 1.62 millimeters. The dilatation had reached its maximum in all cases within one minute after the injection. Within three to five minutes (average, four minutes) after the injection, a recovery of the pupil had begun. Recovery was complete in from five to twenty minutes (average, ten minutes) after the injection.

The quantity of adrenalin (0.02 of a milligram) employed in table I is almost the smallest to which the deganglionized pupil responds. A smaller amount could evidently not be depended upon to produce a dilatation. Control injections of a saline solution

TABLE I.

Adrenalin, 1/50 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (=0.02 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
11	18 hours	0.75 mm.	———	5 minutes
12	18 hours	1.00 mm.	6 minutes	13 minutes
7	46 hours	0.75 mm.	4 minutes	10 minutes
8	46 hours	3.00 mm.	3 minutes	20 minutes
55	51 hours	2.00 mm.	5 minutes	6 minutes
56	51 hours	0.50 mm.	———	5 minutes
9	5 days	2.25 mm.	4 minutes	9 minutes
10	5 days	2.25 mm.	4 minutes	9 minutes
Averages for the last six experiments		1.62 mm.	4 minutes	10 minutes

The averages in this as well as in the four succeeding tables are derived only from the experiments performed twenty-four or more hours after the removal of the superior cervical ganglion.

were made, the same technique being used as for the adrenalin injections, but these caused no dilatation of the pupil.

Adrenalin, 1/30 of a Cubic Centimeter per Kilo of Body Weight.—In this series there were six animals (table II) in all of which the

TABLE II.

Adrenalin, 1/30 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (=0.033 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
19	50 hours	1.50 mm.	7 minutes	7 minutes
20	52 hours	2.00 mm.	5 minutes	11 minutes
54	6 days	3.00 mm.	4 minutes	25 minutes
53	6 days	2.25 mm.	11 minutes	85 minutes
23	19 days	3.00 mm.	10 minutes	20 minutes
22	19 days	1.75 mm.	10 minutes	20 minutes
Averages for all six experiments		2.25 mm.	8 minutes	28 minutes.

ganglion had been removed over twenty-four hours before the injection. The maximum dilatation was observed in three of them within one minute after the injection, while in the three others the widest diameter was recorded only after five minutes. The amount

of dilatation varied between 1.5 and 3 millimeters, with an average of 2.25 millimeters. A beginning recovery from dilatation was noted between four and ten (average eight) minutes, which was complete in from seven to eighty-five (average, twenty-eight) minutes after the injection. There was one marked exception in the time between the injection and complete recovery. In five of the six experiments, recovery was complete in twenty-five minutes or less, whereas in one (rabbit 53) this period was eighty-five minutes in length. Were it not for this exception the average time between the injection and complete recovery would in this series be seventeen instead of twenty-eight minutes.

Adrenalin, $\frac{1}{20}$ of a Cubic Centimeter per Kilo of Body Weight.—There were in this series fourteen experiments, thirteen of which were carried out more than twenty-four hours after gangliectomy (table III). In eleven of the thirteen experiments the dilatation had reached its maximum within one to two minutes after the injection, but in the remaining two it was maximal only after four minutes. It can be seen by a glance at table III that the amount of pupil dilatation produced with this dosage was much more constant than after $\frac{1}{50}$ and $\frac{1}{30}$ of a cubic centimeter of adrenalin,—this being especially

TABLE III.

Adrenalin, 1/20 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (=0.05 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
3	19½ hours	1.25 mm.	6 minutes	13 minutes
6	43 hours	4.25 mm.	5 minutes	30 minutes
5	43 hours	2.50 mm.	8 minutes	39 minutes
1	48 hours	6.00 mm.	8 minutes	80 minutes
2	48 hours	4.00 mm.	11 minutes	75 minutes
33	3 days	3.50 mm.	9 minutes	30 minutes
34	3 days	3.50 mm.	———	50 minutes
15	4 days	3.50 mm.	4 minutes	22 minutes
14	4 days	2.50 mm.	4 minutes	14 minutes
38	5 days	2.75 mm.	6 minutes	20 minutes
13	6 days	3.75 mm.	7 minutes	11 minutes
41	9 days	3.50 mm.	6 minutes	44 minutes
49	14 days	3.25 mm.	4 minutes	26 minutes
28	19 days	3.75 mm.	5 minutes	48 minutes
Averages for the last thirteen experiments		3.60 mm.	6 minutes	37 minutes

evident in those animals in which the ganglion had been removed three days and longer. The maximum dilatation produced varied in the thirteen cases between 2.5 and 6 millimeters, with an average of 3.60 millimeters. Beginning recovery from dilatation was first noted in from four to eleven (average, six) minutes after the injection. Complete recovery had taken place in from eleven to eighty (average, thirty-seven) minutes after the injection. Here again, as with $\frac{1}{30}$ of a cubic centimeter per kilo, the greatest variations (rabbits 1 and 2) occur in rabbits in which the ganglion had been removed forty-eight hours or less.

Adrenalin, $\frac{1}{10}$ of a Cubic Centimeter per Kilo of Body Weight.—In this series there were twelve animals (table IV), in eight of which

TABLE IV.

Adrenalin, 1/10 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (=0.01 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
42	4 hours	No dilatation—definite constriction.		
46	6 hours	No dilatation—definite constriction for 15 minutes.		
44	16 hours	$\frac{1}{2}$ millimeter dilatation followed in 3 minutes by constriction to a size less than normal.		
4	20 hours	3.50 millimeters	6 minutes	70 minutes
21	3 days	3.00 millimeters	6 minutes	28 minutes
30	4 days	4.25 millimeters	7 minutes	40 minutes
52	4 days	3.50 millimeters	5 minutes	38 minutes
32	5 days	4.25 millimeters	20 minutes	40 minutes
16	7 days	2.75 millimeters	7 minutes	53 minutes
17	8 days	4.50 millimeters	4 minutes	46 minutes
18	8 days	4.00 millimeters	19 minutes	65 minutes
48	15 days	4.50 millimeters	10 minutes	55 minutes
Averages for the last eight experiments		3.84 millimeters	10 minutes	45 minutes

the ganglion had been removed more than twenty-four hours before the injection of adrenalin. The time between the injection and the appearance of the maximum dilatation seemed in this series to be somewhat longer than with smaller doses. In three experiments this period was one minute; in one, two minutes; in three, four minutes; and in one, seven (average, three) minutes. In most, if not in all of the animals in which the maximum dilatation appeared late, there were rather marked symptoms produced by the adren-

alin. These symptoms were those of severe prostration, during which the animal's nose usually rested on the table. Occasionally, however, the animal might lie sprawled out for a few minutes, the respiration being deeper and more rapid than normally, the animal taking no interest in its surroundings, and making no attempt to move about. These symptoms were shown in varying degrees by most of the rabbits which received the larger doses; *i. e.*, $\frac{1}{10}$ and $\frac{2}{10}$ of a cubic centimeter per kilo. One rather striking effect of this kind will be mentioned in the account of the next series.

Here, as also with $\frac{1}{20}$ of a cubic centimeter per kilo, the amount of dilatation was fairly constant. It varied in the eight experiments between 2.75 and 4.5 millimeters, with an average of 3.84 millimeters. A beginning recovery was first noted between five and twenty (average, ten) minutes after the injection. Recovery from dilatation was complete in from twenty-eight to sixty-five (average, forty-five) minutes after the injection.

Adrenalin, $\frac{2}{10}$ of a Cubic Centimeter per Kilo of Body Weight.—In this series of eleven animals, a superior cervical ganglion had been removed twenty-four or more hours before the injection of adrenalin, the dose here employed, 0.2 of a cubic centimeter per kilo of body weight, being the largest used in any of the five series (table V). In seven of the eleven experiments the maximum dilatation

TABLE V.

Adrenalin, 2/10 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (= 0.02 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
45	24 hours	5.50 millimeters	5 minutes	38 minutes
43	24 hours	4.00 millimeters	4 minutes	45 minutes
29	4 days	4.75 millimeters	8 minutes	95++ minutes
51	4 days	6.25 millimeters	27 minutes	51++ minutes
31	5 days	4.00 millimeters	30 minutes	110 minutes
39	6 days	4.25 millimeters	14 minutes	170 minutes
36	8 days	3.25 millimeters	13 minutes	76 minutes
37	8 days	4.25 millimeters	8 minutes	40 minutes
35	8 days	3.50 millimeters	41 minutes	145 minutes
40	9 days	5.75 millimeters	43 minutes	170++ minutes
47	14 days	2.50 millimeters	28 minutes	105++ minutes
Averages for all eleven of the experiments		4.36 millimeters	20 minutes	100+ minutes.

was recorded within one to two minutes after the injection. In the remaining four, this period was much longer, being respectively, ten, fourteen, seventeen, and twenty-two minutes in length, so that the average for the eleven animals, six and a half minutes, is considerably longer than in any of the other series with smaller doses of adrenalin.

I shall now describe in detail the conduct of the pupil and the symptoms following the injection of adrenalin into the animal in which the maximum dilatation appeared only after twenty-two minutes.

A white female rabbit, weighing 1,630 grams, received very slowly in an ear vein $\frac{2}{10}$ of a cubic centimeter per kilo of the adrenalin solution. In three minutes the pupil had increased 2.5 millimeters in diameter. It continued to increase for a short time until the total dilatation was 3 millimeters. Then serious symptoms developed. The animal which had up to this time appeared restless, sneezed repeatedly, brushed its nose with its paws, then jumped violently out of the box a number of times. The iris was very pale, and the heart was very rapid. Now and then the symptoms nearly disappeared and the animal sat quietly on its haunches. Respiration was deep and slightly more rapid than normally. During the attack the pupil became smaller and did not again dilate until the animal quieted down, when, twenty-two minutes after the injection, the point of maximum dilatation was reached.

The amount of dilatation produced in this series varied between 2.5 and 6.25 millimeters, with an average of 4.36 millimeters. In only three of the eleven experiments was the maximum amount of dilatation produced less than 4 millimeters, and in two of these it was 3.25 and 3.5 millimeters respectively. The first sign of recovery from dilatation was recorded between four and forty-three minutes after the injection, with an average of twenty minutes. In five of the eleven experiments the maximum dilatation persisted for from twenty-seven to forty-three minutes. This long continued maximum dilatation was more prominent with this dosage than with any other that was used. The return of the pupil to the normal diameter was also far more gradual than after smaller doses of adrenalin. The shortest interval between injection and com-

plete recovery of the pupil from dilatation was thirty-eight minutes, while in one case the recovery was not complete in one hundred and seventy minutes (about three hours). The average length of this interval is over one hundred minutes. It cannot be given accurately, for in several of the experiments the observations were discontinued for various reasons before complete recovery had taken place.

In considering the experiments in each of the foregoing series, I have thus far said nothing regarding those in which the injections were given during the first twenty-four hours after removal of the ganglion. By referring to tables I, III, and IV, and comparing in each table the response of the pupil to adrenalin during the first twenty-four hours after gangliectomy, with the response given at a later time to the same dose of adrenalin, the following points will be noted: Two injections (table IV), made four and six hours respectively after the removal of the ganglion, gave, not a dilatation, but a constriction of the pupil. In another experiment in the same table (rabbit 44), an injection sixteen hours after gangliectomy gave a dilatation of only 0.5 of a millimeter, which was followed in three minutes by a constriction that made the pupil smaller than normally. In still another animal (rabbit 4) an injection given after twenty hours gave a dilatation of 3.5 millimeters; *i. e.*, well up to the average for the whole series of injections given in this table.

In brief, then, these few injections indicate that for the first few hours after removal of the corresponding ganglion, the pupil is no more sensitive to the action of adrenalin than is the pupil of a normal animal; that within sixteen hours after gangliectomy, a faint dilatation may be obtained, while somewhere between the eighteenth and the twenty-fourth hour the sensitiveness rises rapidly to nearly or quite that which is seen at any later time.

The following protocols, one taken from each of the series with different doses of adrenalin, will serve to illustrate the course of the action of adrenalin. As already stated, where two diameters are given for a single pupil, the first is always the horizontal diameter as the animal stands erect, and the second the vertical diameter. In each protocol the pupil on the normal side serves as a control for the one whose ganglion has been removed.

PROTOCOL No. 1.

Rabbit 9. November 23, 1911. White Male; Weight, 1,790 Grams. Right Superior Cervical Ganglion Removed Five Days Ago.

Time.	Size of right pupil in mm.		Size of left (control) pupil in mm.	
10:53	4.0	4.5	4.0	4.5
11:00	4.0	4.5	4.0	4.5
11:05	4.0	4.5	4.0	4.5
11:14	Received 1/50 c.c. per kilo of body weight of adrenalin into left ear vein.			
11:15	6.5		4.0	4.5
11:18	5.5		4.0	4.5
11:23	4.0	4.5	4.0	4.5
11:35	4.5		4.0	4.5
11:56	4.0	4.5	4.0	4.5
12:20	4.0	4.5	4.0	4.5

PROTOCOL No. 2.

Rabbit 23. December 9, 1911. White Male; Weight, 1,400 Grams. Left Ganglion Removed Nineteen Days Ago.

Time.	Size of right pupil (control) in mm.		Size of left pupil in mm.	
10:46	4.0	4.5	3.5	3.75
10:56	4.0	4.5	3.75	4.0
11:05	4.0	4.5	3.75	4.0
11:20	Received 1/30 c.c. per kilo of body weight of adrenalin into right ear vein.			
11:21	4.0	4.5	6.0	
11:24	4.0	4.5	6.5	7.0
11:30	3.75	4.0	5.5	6.0
11:40	4.0	4.5	3.75	4.0
12:00	4.0	4.5	4.5	
12:15	4.0	4.5	4.0	

PROTOCOL No. 3.

Rabbit 2. November 20, 1911. White Male; Weight, 1,315 Grams. Left Superior Cervical Ganglion Removed Forty-Eight Hours Ago.

Time.	Size of right pupil (control) in mm.		Size of left pupil in mm.	
11:10	5.5	6.0	6.0	6.5
11:17	5.5	6.0	6.0	6.5
11:21	5.5	6.0	6.0	6.5
11:35	Received 1/20 c.c. per kilo of body weight of adrenalin into right ear vein.			
11:35	5.5	6.0	10.0	
11:40	5.5	6.0	10.0	
11:46	6.0		9.0	
11:52	6.0	6.5	8.5	
12:00	6.0	6.5	8.0	
12:10	6.0	6.5	7.5	8.0
12:29	6.5		7.0	7.5
12:50	6.0	6.5	6.5	7.0
1:15	6.5		7.0	
1:28	6.0	6.5	6.5	7.0
1:45	6.0		6.5	7.0
2:08	6.0		6.5	7.0
4:15	6.5	7.0	6.5	7.0

PROTOCOL No. 4.

Rabbit 48. January 17, 1912. White Female; Weight, 1,600 Grams. Right Superior Cervical Ganglion Removed Fifteen Days Ago.

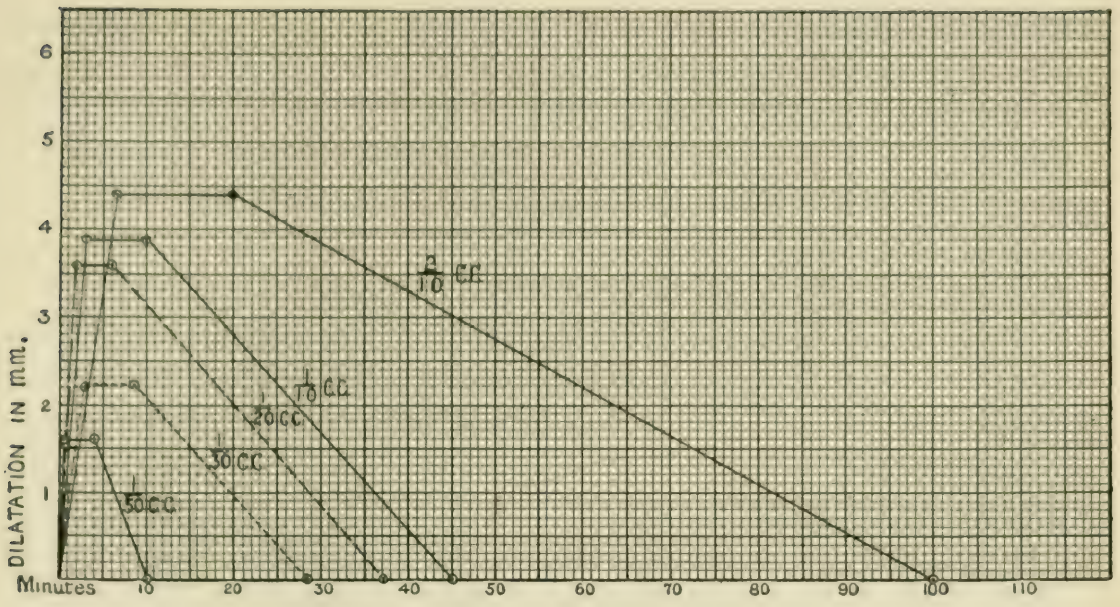
Time.	Size of right pupil in mm.		Size of left (control) pupil in mm.	
1:18	4.75	5.75	5.25	6.25
1:28	5.25	5.75	4.75	5.5
1:33	4.5	5.0	4.75	5.25
1:35	Received 1/10 c.c. per kilo of body weight of adrenalin into left ear vein.			
1:36	7.5	8.5	4.75	5.75
1:39	9.0	9.25	4.5	5.0
1:45	8.0	8.25	4.75	5.25
1:55	7.25	8.0	4.75	5.5
2:07	7.0		4.75	5.0
2:30	5.75	6.0	4.75	5.5
3:13	5.25	5.75	4.75	5.5

PROTOCOL No. 5.

Rabbit 40. January 12, 1912. White Male; Weight, 1,675 Grams. Right Ganglion Removed Nine Days Ago.

Time.	Size of right pupil in mm.		Size of left (control) pupil in mm.	
10:44	3.75	4.25	3.75	4.10
10:52	3.75	4.0	4.5	5.0
10:56	Received 2/10 c.c. per kilo of adrenalin into left ear vein.			
10:57	9.5		3.75	4.5
11:02	8.0	9.0	2.5	3.0
11:07	8.5	9.0	3.5	3.75
11:16	8.0	8.5	3.75	4.0
11:26	9.0		4.0	4.5
11:39	8.0		3.5	4.0
11:51	7.0	7.5	4.0	4.5
12:06	7.0	7.5	4.0	4.5
12:21	7.0		4.5	5.25
12:29	6.5	7.25	4.5	5.25
1:15	5.75	6.25	4.25	5.0
2.04	5.0	5.5	4.0	4.75
3:14	4.75	5.75	4.0	4.5

In the following chart have been plotted the average figures obtained from each series of animals from which one ganglion had been removed twenty-four hours or more before the intravenous injection of adrenalin. The figures show the comparative effects of the different doses of adrenalin upon the pupil. The amount of dilatation is shown on the ordinates, taking the abscissa as the normal diameter before the injection of adrenalin, while along the abscissa is given the time in minutes after the injection.



TEXT-FIG. 1. For description see text.

SUMMARY AND CONCLUSIONS.

It has been shown by these experiments that after the removal of one superior cervical ganglion of a rabbit, $\frac{1}{50}$ of a cubic centimeter of a 1 : 1,000 adrenalin solution (=0.02 of a milligram of adrenalin) per kilo of body weight injected intravenously, is practically the minimum amount that will produce in nearly all cases a perceptible dilatation of the pupil. In one out of eight animals this dose failed to give a definite effect, while in two others the effect was slight.

Six experiments with injections of $\frac{1}{50}$ of a cubic centimeter of adrenalin per kilo of body weight gave the following averages: a maximum dilatation of 1.62 millimeters, which showed the first sign of decreasing in four minutes and had disappeared entirely in ten minutes after the injection.

Six experiments with $\frac{1}{30}$ of a cubic centimeter per kilo gave the following averages: a maximum dilatation of 2.25 millimeters, which began to disappear in eight minutes and had entirely disappeared in twenty-eight minutes.

For thirteen experiments with $\frac{1}{20}$ of a cubic centimeter of adrenalin per kilo these averages were: a maximum dilatation of 3.61

millimeters, a beginning recovery from dilatation in six minutes, with complete recovery of the pupil in thirty-seven minutes.

With $\frac{1}{10}$ of a cubic centimeter of adrenalin per kilo (eight experiments) the averages were: a maximum dilatation of 3.87 millimeters, a beginning recovery in ten minutes, with complete recovery of the pupil in forty-five minutes.

Finally, eleven experiments with $\frac{2}{10}$ of a cubic centimeter of adrenalin per kilo gave averages as follows: a maximum dilatation of 4.38 millimeters, a beginning recovery from dilatation in twenty minutes, with complete recovery only after one hundred minutes or more.

The time between the injection and the appearance of the maximum dilatation of the pupil varied somewhat according to the dose of adrenalin. In the series of experiments with $\frac{1}{50}$ of a cubic centimeter of adrenalin per kilo of body weight the average for this interval was one minute; in the series with $\frac{1}{30}$, $\frac{1}{20}$, and $\frac{1}{10}$ of a cubic centimeter the average length of this interval was about three minutes, while the experiments with $\frac{2}{10}$ of a cubic centimeter give us an average for this interval of six and a half minutes, with individual instances in which the maximum dilatation was seen only after fourteen, seventeen, and twenty-two minutes after the injection.

In those animals in which the maximum pupil dilatation was especially delayed, there were almost always more or less alarming symptoms of general prostration for a short time after the injection, and the maximum dilatation appeared as these symptoms gradually disappeared.

In other words, with an increase in the size of the dose of adrenalin, there was a gradual increase in the following: (1) the time between the injection and the appearance of the maximum dilatation; (2) the amount of dilatation produced; (3) the interval between the injection and the beginning of recovery from dilatation; and (4) in the total time between the injection and the return of the pupil to its normal size.

After removal of the ganglion a certain time must elapse before the increase in the sensitiveness develops. During the first ten to fifteen hours there is practically no increase in the sensitiveness. After eighteen hours a moderate effect can be obtained which rap-

idly increases so that by twenty to twenty-four hours after the removal of the ganglion any given dose of adrenalin produces practically as great a dilatation as it will give at any later time.

The experiments teach us that, on account of the individual variation in the degree of dilatation produced by a given dose of adrenin, we may not determine with exactness from any given degree of dilatation of the pupil the quantity of adrenin injected into the blood stream, and that we can not state with absolute exactness either the smallest dose that will constantly cause a dilatation, or mention the exact amount of adrenin which will in no case cause a dilatation of the pupil. However, the results permit the general statement that very small doses of adrenin on entering the circulation cause a fairly prolonged definite dilatation of a deganglionized pupil.

Since this is true, there are then at least two ways in which a rabbit from which one superior cervical ganglion has been previously removed may be used for determining qualitatively the amount of adrenin present. If, for example, we have a solution which may or may not contain adrenin, and upon injecting it obtain a dilatation of the deganglionized pupil only while the pupil on the normal side remains unchanged in size, we are justified in assuming that the solution contains adrenin. Again, if after stimulation of the peripheral end of a splanchnic nerve or other procedure upon the animal body under conditions that exclude the possibility of reflex effects, a dilatation of the deganglionized pupil results, while its normal mate remains unchanged, the assumption is warranted, though not absolutely proved, that some adrenin has been thrown into the circulation.

This test for the presence of adrenin has the advantage over other tests, that the pupil on the normal side will always act as a control.

TRANSPLANTABLE TUMORS OF THE FOWL: A NEGLECTED MATERIAL FOR CANCER RESEARCH *

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The recognition that neoplasms exist in many species of animals throughout the vertebrate world has given to the tumor problem a broader biologic significance. And at first glance it appears strange that so few animal forms have been utilized for experimental cancer research. Attention has centered on the new growths of the rat and mouse, on a peculiar round-celled tumor of the dog, transmissible by coitus, a dubious tumor of wild hares, endemic in certain portions of Germany, and on a thyroid growth of fishes, the exact nature of which is still under dispute. There exist good reasons for this limitation. The species employed for tumor work must be available in quantity and capable of thriving under laboratory conditions; and its "spontaneous" growths must be transplantable. True tumors are rare in cats, rabbits and guinea-pigs, and, while more frequent in dogs, are difficult to study because of the number of individuals which must be employed and the frequent necessity that all shall be of one age and breed.

Some two years ago one of us transplanted a spindle-celled sarcoma of the fowl, a very typical neoplasm, metastasizing by means of its cells, and in other ways regulated by their peculiarities. The growth has since been observed in twenty-seven series of fowls. Recently it has been successfully transmitted by the Berkefeld filtrate of an extract of the fresh tumor-tissue in Ringer's solution[1] and by the dried or glycerinized tissue. These results have led us to a study of other chicken-

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. Rous, Peyton: Jour. Exper. Med., 1910, xii, 696; 1911, xiii, 397; THE JOURNAL A. M. A., 1910, lv, 1805; 1911, lvi, 198. Rous, P., and Murphy, James B.: Jour. Exper. Med., 1912, xv, 119, 270.

tumors. Work with them has not yet advanced to tests for an extraneous causative agent; but it has disclosed the presence in chickens of an abundant material well adapted for cancer research. From a single firm selling live poultry in New York we have acquired during the past seven months twenty-seven chicken-tumors, a number greater than this institute was able to obtain of mouse-tumors from all sources in the course of three years.[2] Furthermore, the avian growths were encountered in a relatively unfavorable stock, young for the most part and supposedly healthy.

The existence of tumors of the fowl has been demonstrated by the work of several investigators.[3] The variety of growths is much greater than among rats and mice. We have been especially struck with the frequency of benign tumors. Among 353 primary neoplasms of the mouse, Haaland[4] found but three which may be termed benign — a melanoma, a mixed tumor and a fibromyoma uteri. There were 344 carcinomas and six sarcomas.[5] But among our first twenty chicken-tumors were a large, characteristic lipoma, a pure myxoma, and several stationary growths, made up of blood-, or lymph-sinuses and lymphoid tissue, which are perhaps best thought of as nevi. Of malignant tumors there were an osteosarcoma with large blood-sinuses, from one of which the fowl bled to death at operation, numerous round-celled tumors, a connective-tissue growth producing cartilage and bone, three spindle-celled sarcomas, a fibrosarcoma and an epithelioma. Previous observers have noted sarcomata and carcinomata in about equal proportions. But they have dealt in large part with autopsy material and with growths primary in the viscera; whereas our records have to do with tumors superficially situated, and for this reason come to hand during the life of the host.

Small bits of each spontaneous growth were removed at operation and transplanted by means of a trochar. The selection of a vascular site for the grafts has much importance. Usually they were placed deep in the pec-

2. Jobling, J. W.: Monographs of the Rockefeller Institute for Medical Research, 1910, No. 1, p. 81.

3. Ehrenreich and Michaelis, Tyzzer, Wernicke.

4. Haaland, M.: Fourth Scientific Report of the Imperial Cancer Research Fund, 1911, p. 1.

5. Haaland excluded from these figures thirty of the lung-adenomas peculiar to mice and twenty-five lymphomas, because of uncertainty as to their significance.

toral muscle. The normal fowls taken as hosts were young, and of the same variety as the chicken with the spontaneous growth. The general failure to transplant avian growths is certainly attributable, in part, to lack of observance of such points.[6]

Many attempts were made to transplant the first tumors obtained, quite irrespective of the indications afforded by their histologic appearance. More recently, taught by negative results, we have resorted to preliminary autotransplantations, and have tried to propagate further only such growths as proved autotransplantable, or were found micoscopically to be malignant.

On much of the work it is too early to report. But thus far two new tumors have been successfully transferred to other hosts. One of these, a connective-tissue growth forming cartilage and bone, is noteworthy, because in the original host it appeared to be a symmetrical, developmental anomaly connected with the sternum. It is now in its fifth "tumor-generation." The other growth is a spindle-celled sarcoma which had its primary seat amid the muscles of the upper leg, but metastasized to the neck and gizzard. It is now growing rapidly in a large proportion of the fowls constituting the second "tumor-generation" (second consecutive series inoculated).

Of special interest is the question as to whether our spindle-celled sarcoma with a filterable cause is epidemic among chickens. But in the new material it has not even been encountered. True, several spindle-celled sarcomas have been observed, including one that is transplantable. But each has its distinguishing histologic features. There remains the possibility, nevertheless, that these various growths may have a common cause, a point which we hope to investigate in the near future.

Sixty-Sixth Street and Avenue A.

6. Rous: Note 1. From a recent publication (Verhandl. d. Japan patholog. Gesellsch., 1911, p. 114) it appears that A. Fujinami and K. Inamoto reported in Osaka, in 1910, their success in transplanting a myxoma of the fowl. They make no mention of the influence of variety of the host on the success of transplantation.

Reprinted from The Journal of the American Medical Association
June 1, 1912, Vol. LVIII, pp. 1682 and 1683

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Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XI. No. 5, 1912.

ON SPHINGOSINE.[1]

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(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

(Received for publication, May 2, 1912.)

Sphingosine was discovered by Thudichum[2] on hydrolysis of a cerebroside, phrenosine. Discussing the chemical properties of the substance, its behavior towards bases and acids, the author took into consideration the possibility of the substance having the structure of an amino-acid or of an alkaloidal base. In his final conclusion he expressed preference to the view of the basic nature of the substance.

In later years Thierfelder[3] repeated the work of Thudichum, and in the main substantiated his views. The work of Thierfelder, however, was directed principally to the study of the properties of

[1] A report on the results of the present investigation has appeared in the Proceedings of the Meeting of the American Society of Biological Chemists, held December 28th to December 30th, 1911, published in the March number of this Journal. In Heft 6, vol. lxxvii of Hoppe-Seyler's *Zeitschrift für physiologische Chemie*, published on April 9, there appeared two articles by Thierfelder, Riesser and Thomas in which the authors arrived at the same conclusions as reported by us. The appearance of the two articles was caused undoubtedly by the publication of our report, since Heft 5 of vol. lxxvii of Hoppe-Seyler's *Zeitschrift* contained no mention of Thierfelder's name among the authors of twenty-six articles received for publication. In a footnote to one of the articles Professor Thierfelder claims the sole privilege for work on sphingosine and related substances, for the reason that Thudichum's work had been half forgotten at the time when Thierfelder directed his attention to cerebrosides. We do not feel that this justifies the request made by Thierfelder, that the work which had been in progress in our laboratory for more than a year should be abandoned before it is completed.

[2] *Die chemische Konstitution des Gehirns*, Tübingen, 1901.

[3] *Zeitschr. f. physiol. Chem.*, xliv, p. 366, 1905; Kitagawa: *Ibid.*, xlix, p. 286, 1906.

the cerebroside, which he named "cerebron." The author expressed no definite view regarding the chemical structure of the base.

The results of the present investigation have made certain that sphingosine is an unsaturated monoaminodihydroxyalcohol.

This conclusion is based on the following data:

1. The substance contains all its nitrogen in form of primary amino nitrogen.

2. The presence of a double binding in the molecule is demonstrated by the readiness with which sphingosine absorbs hydrogen when treated according to the method of Paal. A substance is thus formed which has the composition of dihydrosphingosine. It was analyzed in the form of a sulphate and a triacetylderivative.

3. The presence of two hydroxyl groups in the molecule is evident from the fact that sphingosine forms a triacetylderivative which no longer contains the original primary amino group. The substance forms a dimethylether, and finally it can be reduced to an amine, sphingamine.

As yet it is not certain whether or not the carbon atoms are linked in a normal chain. Attempts were made to reduce the dihydrosphingosine to the corresponding amine, but instead of the heptadecylamine there was always obtained the unsaturated sphingamine. Efforts to obtain the saturated amine are now in progress. Also work is in progress on the respective position of the double bond, and of the hydroxyl groups.

EXPERIMENTAL PART.

Sphingosine.

The base was obtained on hydrolysis of "cerebrin" prepared by a slight modification of the process described by Parcus.[4] The conditions of hydrolysis were similar, but not identical with those described by Thierfelder. The base was prepared in crude form as the sulphate, which was then transformed into the free base and into the acetate. The discoverer of the base mentioned that it could be made to crystallize out of ether. It was found in course

[4] *Journ. f. prakt. Chem.*, xxiv, p. 310, 1881.

of this work that crystallization proceeded much more readily out of petrolic ether.

The sulphate was obtained in the form of a white crystalline powder. It melted with decomposition at 233° to 234°C. (uncorrected). A great many samples were analyzed. The analysis of one of these gave the following results:

0.0996 gram of the substance dried in chloroform-vacuum bath over phosphorus pentoxide gave on combustion 0.0940 gram of H_2O and 0.2280 gram of CO_2.

0.2400 gram of the substance, employed for a Kjeldahl nitrogen estimation, required for neutralization 6.9 cc. of $\frac{N}{10}$ acid.

	Calculated for $(C_{17}H_{35}NO_2)_2H_2SO_4$:	Found:
C	61.08	61.05
H	10.78	10.60
N	4.19	4.06

The optical activity of the substance was the following:

0.5304 gram of the sulphate was dissolved in a mixture of 5 cc. of chloroform and 1 cc. of glacial acetic acid. The total weight of solution was 8.7514 grams. The rotation in pure D-light was −1.50°, hence

$$[\alpha]_D^{20} = -13.12° \ (\pm 0.00).$$

Diacetate. Dissolved in glacial acetic acid and petrolic ether, the substance crystallized in form of very long needles of the following composition.

0.1402 gram of the substance gave on combustion 0.1290 gram H_2O and 0.3206 gram of CO_2.

	Calculated for $C_{17}H_{35}NO_2.(C_2H_4O_2)_2$:	Found:
C	62.22	62.36
H	10.61	10.22

Amino nitrogen estimation. A solution of 0.300 gram of the sulphate in 10.0 cc. of glacial acetic acid was employed for an amino nitrogen estimation according to the method of Van Slyke. Five cc. were used for each experiment. All nitrogen was given off in thirty minutes. In each experiment 11 cc. of nitrogen were formed at $t = 21°C.$ and $p = 760$ mm.

	Calculated for $(C_{17}H_{33}O_2.NH_2)_2H_2SO_4$:	Found:
N	4.19	4.17

Dihydrosphingosine.

The hydrogen absorption value of sphingosine is obtained most conveniently when the free base is dissolved in ether and shaken with aqueous colloidal palladium prepared according to Paal. 0.100 gram of palladium to about 0.500 gram of the base dissolved in about 150 cc. of absolutely pure ether gave the most satisfactory results. The absorption was completed in about thirty minutes. The velocity of the operation was greatly increased by the addition of 1 cc. of glacial acetic acid to the ethereal solution.

0.500 gram of the substance absorbed 50 cc. of hydrogen (without correction for t and p). Theory requires 45 cc. of H.

0.6486 gram of the substance absorbed 59 cc. of H; theory requires 56 cc.

The ethereal solution of dihydrosphingosine was evaporated to dryness and the substance converted into the sulphate and into the triacetylderivative.

The sulphate was obtained in form of a white crystalline powder. Its melting point was only slightly different from the unsaturated compound, being 235°C.

	Calculated for $(C_{17}H_{37}NO_2)_2H_2SO_4$:	Found
C	60.61	60.90
H	11.38	11.11

The optical activity of the substance was difficult to determine for the lack of a sufficiently satisfactory solvent. Approximately it was as follows:

0.0776 gram of the substance dissolved in about 3 cc. of alcohol containing sulphuric acid, and weighing 2.8640 grams gave a rotation of −0.29° in a 2 dm. tube.

$$[\alpha]_D^{20} = -10.67°.$$

0.1214 gram of the substance gave on combustion 0.1153 gram of H_2O and 0.2948 gram of CO_2.

	Calculated for $C_{17}H_{34}NO_2.(CH_3CO)_2$:	Found:
C	66.76	66.76
H	10.50	10.60

Acetylderivatives.

On treatment of the free base with acetic anhydride di- or triacetylsphingosine can be obtained. The first is obtained by dissolving the base in boiling acetic anhydride and evaporating the solution under diminished pressure. The triacetylderivative is prepared by allowing the base to digest with acetic anhydride in a boiling water bath with return condenser for one hour and only then evaporating the solution to dryness. The further treatment in both instances is identical. The residue obtained on evaporating the solution under diminished pressure is taken up in chloroform and again evaporated under diminished pressure. This residue is taken up in hot acetone and the substance allowed to crystallize. For analysis the substances were dried in a vacuum-chloroform bath over phosphorus pentoxide.

Diacetylderivative. 0.1317 gram of the substance gave on combustion 0.1256 gram of H_2O and 0.3283 gram of CO_2.

0.2720 gram of the substance was dissolved in 10 cc. of glacial acetic acid and used for amino nitrogen estimation according to Van Slyke. Five cubic centimeters of the solution were employed for each experiment. There was formed 9.3 cc. nitrogen at 24°C. and 758 mm. pressure. The substance was allowed to react one hour, although the reaction was practically completed in twenty minutes.

	Calculated for $(C_{17}H_{31}O_2.NH_2).(CH_3CO)_2$:	Found:
C	68.1	68.16
H	10.8	10.59
N	3.4	3.78

The physical constants and saponification value of this substance were not determined.

Triacetylderivative. 0.1194 gram of the substance gave on combustion 0.1064 gram of H_2O and 0.2950 gram of CO_2.

0.2500 gram of the substance was dissolved in 10 cc. of glacial acetic acid and employed for an amino nitrogen estimation. No formation of nitrogen took place.

	Calculated for $C_{17}H_{32}NO_2.(CH_3CO)_3$:	Found:
C	67.15	67.38
H	9.98	9.98

The substance melted sharply at 102° to 103° C. (uncorr.).

0.3383 gram of the substance was dissolved in 60 cc. of methyl alcohol, containing 10 cc. of a $\frac{N}{2}$ solution of sodium hydrate in methyl alcohol. The solution was heated on boiling water bath for two hours, allowed to stand over night and titrated. It required 24.90 cc. of $\frac{N}{10}$ alkali to neutralize the acetic acid formed on saponification. The theory required 24.75 cc.

Dimethylsphingosine.

This substance is formed in course of hydrolysis of cerebrosides by means of methylalcohol and mineral acid. Thierfelder, who was the first to have the substance in his hands, erroneously regarded it as a new base. Since our first communication, Thierfelder and Riesser[5] substantiated our view on the substance. The methylderivative was obtained in form of a sulphate on concentrating the mother liquors from the crude sphingosine sulphate. The sulphate was then transformed into the free base and this again transformed into the hydrochloride. The hydrochloride crystallizes out of alcohol in the form of large glittering plates.

The substance was identified by the fact that, similarly to sphingosine, it contained all its nitrogen in form of primary amino nitrogen; it contained one unsaturated bond and on boiling with hydroiodic acid formed the required amount of methyliodide.

0.1615 gram of the substance gave on combustion 0.1654 gram H_2O and 0.3926 gram of CO_2.

	Calculated for $C_{19}H_{39}NO_2.HCl$:	Found:
C	65.18	66.65
H	11.52	11.24

Hydrogen absorption value. One gram of the substance dissolved in ether containing 2 cc. of glacial acetic acid. On treatment with palladium according to Paal it absorbed 67 cc. of hydrogen. Theory requires 72.6 cc.

Amino nitrogen estimation. 0.3500 gram of the hydrochloride dissolved in 10 cc. of glacial acetic acid. Five cc. of this solution used for amino nitrogen estimation according to Van Slyke. There formed 12.5 cc. of nitrogen at $t = 24°$ and $p = 758$ mm.

	Calculated for $C_{19}H_{39}O_2.NH_2HCl$:	Found:
N	4.01	4.00

Methyl estimation. 0.1206 gram of the hydrochloride boiled with hydroiodic acid of specific gravity = 1.71 in the apparatus of Zeisel and Fanto. There was obtained 0.1286 gram of silver iodide.

	Calculated for $C_{17}H_{33}NO_2.(CH_3)_2.HCl$:	Found:
CH_3	8.45	7.00

The physical properties of the substance did not permit a more accurate estimation. In a control experiment with sphingosine

[5] *Zeitschr. f. physiol. Chem.*, lxxvii, p. 508, 1912.

crystallized out of petrolic ether no silver iodide was formed. On the other hand, the base obtained directly after removing the sulphuric acid from apparently pure sphingosine sulphate still caused the formation of some silver iodide. The highest value obtained in this manner was equivalent to CH_3 = 2.32 per cent.

Sphingamine.

Attempts were made to reduce dihydrosphingosine to the corresponding amine. The normal heptyldecylamine has been obtained synthetically. Hence a comparison of the two bodies should have determined the fact whether or not the substances were identical. In several experiments the reduction was attempted by means of hydroiodic acid, and in one experiment the dihydrosphingosine was transformed into the dihydrodichlorsphingosine, which was then reduced by means of metallic sodium and alcohol.

However, under all conditions the unsaturated substance was formed.

Reduction with hydroiodic acid was carried out in sealed tubes at 125°C. The reaction-product was dissolved in ether. The ethereal solution was dried with anhydrous sodium sulphate, and then diluted with one-third of its volume of 98 per cent alcohol, and the solution treated with metallic sodium.

The substance obtained from the solution was transformed into the sulphate. The analysis of the substance obtained from three different experiments follows:

I. 0.1042 gram of the substance gave 0.1100 gram H_2O and 0.2562 gram of CO_2.

II. 0.1014 gram of the substance gave 0.0998 gram H_2O and 0.2494 gram of CO_2.

III. 0.1153 gram of the substance gave 0.1233 gram H_2O and 0.2800 gram of CO_2.

0.1480 gram of sample I was used for Kjeldahl nitrogen estimation. It required for neutralization 4.1 cc. of $\frac{N}{10}$ acid.

	Calculated for $(C_{17}H_{34}N)_2H_2SO_4$:	Found:		
		I	II	III
C	67.46	67.2	67.2	67.85
H	12.02	11.82	11.05	11.97
N	4.52	4.44	4.51	

Reduction of dihydrodichlorsphingosine. The chlorderivative was obtained by digesting dihydrosphingosine with thionylchloride in a water bath at 50°. The crude substance without purification was dissolved in a mixture consisting of two parts of ether and one of 98 per cent alcohol and reduced with metallic sodium. The substance obtained in this manner was transformed into the sulphate and analyzed.

0.1251 gram of the substance gave on combustion 0.1310 gram of H_2O and 0.3097 gram of CO_2.

	Calculated for $(C_{17}H_{35}N)_2H_2SO_4$:	Found:
C	67.46	67.51
H	12.02	11.72

ON THE TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING. A STUDY UPON SERUM HEMOLYSINS IN GOATS

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Reprinted from
THE JOURNAL OF INFECTIOUS DISEASES, Vol. X, No. 3, May 1912, pp. 332-368

CHICAGO

ON THE TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING. A STUDY UPON SERUM HEMOLYSINS IN GOATS.*†

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OUTLINE.

I. Introduction: The Inheritance Problem; Its Relation to Disease and Immunity; Fundamental Studies; Conclusions.

II. General Review of Work upon the Subject of Transmission of Immunity from Mother to Offspring.

A. Antibodies of Well Differentiated Types.

1. *Antitoxins induced by:*
   a) Plant toxalbumins: (1) Abrin; (2) Ricin; (3) Robin.
   b) Bacterial, extracellular toxins: (1) Tetanus; (2) Diphtheria; (3) Pyocyaneus; (4) Symptomatic anthrax; (5) Vibrio Nasik.
   c) Animal venoms.
   d) Ferments.
2. *Agglutinins induced by:*
   a) Bacteria: (1) Typhoid (clinical observations and animal experimentation); (2) Tuberculosis (clinical observations); (3) Cholera; (4) Proteus.
   b) Blood. (1) Sheep corpuscles.
3. *Precipitins induced by:*
   Sera.
4. *Cytolysins induced by:*
   a) Bacteria.
   b) Blood corpuscles.
5. *Opsonins induced by:*
   Bacteria.
6. *Allergins induced by:*
   Sera.

B. Antibodies of Poorly Differentiated Types.

1. *Bacterial, intracellular toxins:*
   a) Organisms: (1) Anthrax; (2) Swine erysipelas; (3) Tuberculosis; (4) Dysentery.
2. *Spirilla:*
   a) *Sp. Obermeieri.*
   b) *Sp. pallida.*
3. *Protozoa:*
   b) Piroplasmosis (dog).
   b) Rabies.

* Received for publication February 19, 1912.

† This work was aided by a grant from the Rockefeller Institute for Medical Research.

4. *Ultramicroscopic organisms:*
a) Sheep-pox (ovine).
b) Foot and mouth disease.
c) Small-pox and vaccinia.

III. DISCUSSION OF RESULTS.

IV. REPORT UPON ADDITIONAL WORK CONCERNING TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.
1. Introductory remarks: factors in passive transmission of antibodies; group of so-called intracellular bacterial toxins; immunity against intracellular toxins; complexity of this immunity process; cytolytic bodies as index to serum value; use of hemolysins in such studies.
2. Review of work reported upon transmission of hemolytic antibodies.
3. Choice of experimental animals and outline of technic.
4. Groups of experiments:
a) Active immunization of the adult before birth of the young, either during the period of gestation or before conception had taken place.
Series 1. Preliminary experiments.
Series 2. Special experiments.
b) Active immunization of the adult following immediately or shortly after the birth of the young.
Series 3. Animals normal at time of parturition.
Series 4. Animals partly immunized at time of parturition.
c) Active immunization of the newly born or older kid against foreign blood cells.
Series 5. Blood injections into kids.
d) Gastro-intestinal absorption of antibodies.
Series 6. Feeding of hemolytic serum to newly born kids.
5. General discussion of results.
6. Summary.

BIBLIOGRAPHY.

1. INTRODUCTION.

The inheritance, by the progeny, of newly acquired biological qualities from either or both parents has been a question of long standing. It has attracted the attention of numerous investigators, particularly physicians, since the beginning of scientific medicine and biology. Certain tendencies to disease were considered to be transmissible from parents to offspring (inherited). On the other hand, observations were recorded of cases where the mother, shortly after having recovered from a disease, gave birth to a child, and the child upon exposure to the same disease showed resistance against the infection. As an example of this character may be mentioned variola (Lereboullet, Buchner).[1] Burckhardt[2] made similar observations upon infants born from mothers

vaccinated (vaccinia) during late pregnancy. In small-pox an intrauterine infection of the fetus may occur, in which case not a true inherited, but an actively acquired, resistance results in the child; possibly the same thing occurs in vaccinia. The transient resistance which infants commonly show against many infections during the first weeks of life, while not fully understood, is probably for the most part of maternal origin.

As is evident, the problem has been beset with difficulties, and very little progress was made toward a solution until the newer discoveries in immunity became known. Immunity actively acquired against specific infectious agents or certain toxins offered itself as a means, readily applicable, by which an insight might be gained concerning the general laws of inheritance, and at the same time afford practical data on the subject of immunity. Following the discovery of the exciting causes of many of the infectious diseases, and the isolation of these specific organisms, animal experimentation was undertaken by several investigators. Chauveau,[3] in 1880, found that by vaccinating with anthrax organisms the slightly susceptible Algerian sheep, during gestation, their offspring were born immune against this organism. These results were supported later by other workers using symptomatic anthrax (Arloing, Cornevin, and Thomas,[4] Kitasato[5]).

A means had now been acquired by which fairly definite tests could be employed under conditions subject to control. More exact methods were introduced for actively immunizing animals against various antigens. The resulting degree of immunity became subject to standardization, thus establishing a firmer basis for studying the problem. Subsequently much important work appeared which threw more light upon the question.

The publication, in 1892, of the results of Ehrlich's[6] basic studies marked a new epoch in the development of the subject. He carried out immunization experiments by feeding mice the toxalbumins, abrin, ricin, and robin. It was found that the young from immune mothers acquired an immunity, which he considered as passive, that is, a passage over of the specific antibodies to the fetus before birth, and then through the milk to the nursling after birth. He proved by placing normal young upon immune nurses, that the milk transmitted specific antibodies which were absorbed by the

nurslings, and caused their immunization. Also, he found that highly immunized males, when crossed with normal females, failed to endow the young with resistant powers against the specific toxin, and so concluded that the idioplasm of the sperm did not carry over immunity to offspring.

In opposition to Ehrlich's assertion that the immunized male alone was unable to transmit an acquired immunity to his descendants, several workers brought forward experiments which conflicted with his results. Among these workers may be mentioned Gley and Charrin,[7] Tizzoni and Cattani,[8] and Tizzoni and Centanni.[9] Ehrlich and Hübener[10] (1894) repeated similar experiments, using tetanus toxin against both guinea-pigs and mice, with results which sustained Ehrlich's first findings. In a critical review of the opposing work, they pointed out certain experimental conditions in each case which they considered invalidated the results. The investigations of Wernicke[11] (1895), Vaillard[12] (1896), and others (Remlinger,[13, 65] Dieudonné,[14] Bulloch,[15] etc.) fully supported Ehrlich's findings. So at the present time the view is, for the most part, generally accepted that acquired immunity is not inherited by the offspring in the ontogenetic sense. In the mammalia apparently the germ plasm of neither parent plays an appreciable rôle in transmitting an acquired immunity to young.*

In discussing the question of the transmission of an acquired immunity from parents to the offspring, attention must be directed to the mother. The specific immunity of the offspring is a passive condition, and not a biological inherited condition of long standing. Therefore the subject may be transferred from the province of biological heredity (germ plasm) to the field of practical medicine and prophylaxis, and be considered from that standpoint.

II. GENERAL REVIEW OF WORK UPON THE SUBJECT OF TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

An extensive literature pertaining to the transmission of immunity from mother to offspring has developed in the past two decades. Since I have had occasion to make a rather exhausted

* It may be of interest to mention in this connection the work of Klemperer (*Arch. f. exper. Path. u. Pharmacol.*, 1893, 31, p. 356), who showed that the yolk of eggs from hens which had been actively immunized against tetanus showed antitoxin. Dzierzgowski (*Centralbl. f. allg. Path. u. path. Anat.*, Ref., 1901, 12, p. 715) confirmed these results with hens actively immunized against diphtheria. He states that chicks hatched from such eggs showed diphtheria antitoxin in their blood serum.

review of this literature, perhaps it may be of interest if a brief outline of the more important papers dealing with the subject be submitted. Special consideration will be given those papers which are important either from a historical standpoint, or those of fundamental value in the development of the subject. No attempt will be made to abstract even briefly the numerous articles, but instead a provisional classification of antibodies and antigens will be outlined, and under these headings the references will be catalogued. These papers, in general, concern the transmission of specific antibodies to the young by actively immunized animals. In some instances, simply the presence of antibodies in the milk of such actively immunized animals are reported. Purposely the work upon passive immunization of the mother animal is omitted, since in our studies attention is directed only to actively immunized mothers and their offspring.

A. Antibodies of Well Differentiated Types.

1. *Antitoxins.*—Animal experimentation has been carried out upon the following subgroups of toxins: *a*) Plant toxalbumins. As mentioned earlier, animals immunized against abrin, ricin, and robin transmitted the antibodies to their own young, o to normal sucklings (Ehrlich[6]).

b) Bacterial, extracellular toxins. (1) Tetanus. Actively immunized rats and rabbits (Tizzoni and Cattani[16]), guinea-pigs and mice (Ehrlich and Hübener[10]), and mare (Ransom[17]) transmitted an immunity to their offspring. It is of historical interest that Brieger and Ehrlich,[18] in a study upon antitoxin-bearing milk from goat immunized against tetanus, published the first immunity curves. (2) Diphtheria. In diphtheria, clinical cases have been reported in which mothers recovering from the disease have apparently transmitted antitoxins to their infants (Kayser,[19] and others). But it has been shown that a high percentage of infants from normal mothers show antitoxic properties in their sera after birth; therefore the interpretation of the above observations are open to question (Fischl and v. Wunschheim[20]). Among animal observations, guinea-pigs (Wernicke,[11] Anderson[21]) and mare (Salomonsen and Madsen[22]) have been shown to transmit immunity or antibodies against diphtheria to their young. (3) Pyocyaneus. In some cases rabbits (Charrin and Gley[23]), when immunized during the course of pregnancy, transmitted, to a certain degree, specific protective powers to their young. (4) Symptomatic anthrax. As mentioned, different workers have found that a certain degree of immunity is transmitted from the recently immunized mother to her young (Arloing, Cornevin and Thomas,[4] Kitasato[5]). (5) Vibrio Nasik (vibriolysin). Wegelius[24] reports that goats and rabbits, when immunized either shortly before conception or during gestation, transmit the specific antibodies to their young.

c) Animal venoms. Fraser[25] states that a cat which was immunized against snake venom during the course of pregnancy gave birth to young which suckled mother; one kitten, when given two minimum lethal doses (subcutaneously) on the 57th day, showed marked resistance against the venom with only very slight symptoms,

from which it soon recovered; the other kitten on the 60th day was given three fatal doses of venom from which it failed to survive.

d) Ferments. Morgenroth[26] immunized goats against rennin and found in a study of the milk that the specific antibody was present in considerable amounts. No example was found in the literature where studies were made upon the transmissibility of antibodies of this type from mother to young.

2. *Agglutinins.*—Including *a*) bacterial, and *b*) hemagglutinins. *a*) Bacterial agglutinins. (1) Typhoid. In view of the fact that much work has been done upon this group of agglutinins both (*a*) clinically (patients) and (*b*) experimentally (animals), a brief outline of the two subgroups will be given. (*a*) Among the clinical observations of possible intrauterine transmission of typhoid agglutinins from mother to fetus, a number of cases have been reported in which the mother aborted during the course of the disease. The body fluids from fetuses ranging in age from three to six months failed to show specific agglutinins (Etienne,[27] Charrier and Apert,[28] Dogliotti[29]). However, Scholtz[30] reported a strong agglutinating reaction from fluids of a seven-months' fetus. In other cases (Schumacher,[31] Stäubli,[32] Mosse and Daunic[33]) blood, taken from the fetal end of cord at birth (about or full term) of children born from mothers who had suffered during the course of pregnancy from typhoid, gave positive reactions. Other cases are reported (Castaigne,[34] Courmont and Cade[35]) in which infants, taking the milk from mothers who were infected as late as two months (one case) after having given birth to young, also showed positive agglutinating sera. However, this was not always the case, since in a few instances (Kasel and Mann[36]) the milks (2) were positive (1:25)—the blood agglutinated 1:50—but no reaction was given by the suckling's sera. The mothers in this series were infected 2–21 years before parturition. In another instance (Achard and Bensaude[37]) a mother contracted the disease while nursing the child (age ?); the milk showed a low agglutinating value (1:10); the blood of the nursling was negative. In the other cases the milk from the mother when tested showed a positive reaction, with few exceptions. (*b*) The published studies upon animal experimentation add nothing further to the results already cited. Widal and Sicard[38] reported positive results in young rabbits born from a mother which was inoculated with typhoid bacillus six days before parturition. The heart blood taken from young at time of birth, however, was much weaker in agglutinating power than the mother's blood. Guinea-pigs actively immunized during pregnancy in most cases transmitted agglutinins to their young (Remlinger,[13] Jurewitsch,[39] Stäubli[40]). It is noteworthy that a goat which was highly immunized late in pregnancy gave birth to a kid whose blood showed no specific agglutinins (Schumacher[31]). This kid had not suckled before a blood sample was taken.

Among other organisms whose specific agglutinins have been reported as passing to the young from the actively immunized mothers, the following may be mentioned: (2) *B. tuberculosis;** specific agglutinins passed from infected mother to child (Lagriffoul and Pages[41]). (3) Cholera; specific agglutinins were transmitted from guinea-pigs immunized during pregnancy to the fetus or the young (Achard,[42] Dieudonné[14]). (4) Proteus; specific agglutinins were found in the serum of young guinea-pigs from immunized mothers (Achard[42]).

Specific agglutinins for the above and other microorganisms have been demonstrated in the milk of immunized animals. But, as is evident, it does not follow that agglutinins are transmitted with any regularity from mother, either intrauterine or

* Figari (*Centralbl. f. Bakt.*, I, Ref., 1907, 39, p. 75), reports that hens fed upon tubercle bacillus preparations after a time showed specific agglutinins in their eggs.

through the milk, to the offspring. For instance, a goat showing a reaction of 1:400 against anthrax failed to pass much agglutinin over to the still-born young; the kids' sera reacted in 1:10 dilution, which was much less than was normally present in the mother's blood before inoculation (Gengou[43]). (*b*) Hemagglutinins. Kraus[44] reported that transmission of immune hemagglutinins did not take place in a kid born from a mother which had been immunized against red blood cells of sheep, but these immune bodies were demonstrated in the goat's milk. In infant's blood serum (cord) at time of birth, natural hemagglutinins were found to be less in amount than in the mother's serum (Halban,[45] Halban and Landsteiner[46]), although there was much variation, depending upon the source of the test corpuscles.

3. *Precipitins.*—Specific precipitins against human blood serum have been reported by Merkel[47] in the serum of young rabbits (blood taken shortly after birth) from mothers which were immunized during the period of gestation. He considers that it was a case of placental transmission. Bertarelli[48] immunized two dogs by subcutaneous injections of horse serum and of cow serum respectively; later he found a small amount of precipitins in the milk of each but found none in the blood serum of their young after four weeks nursing.

4. *Cytolysins.*—*a*) Bacteriolysins. Normal bacteriolytic bodies have been frequently demonstrated in different milks; likewise specific bacteriolytic substances have been demonstrated in the milk of immunized animals (Kraus[49]). No experimental work has been found demonstrating a transmission of specific bacteriolysins from the mother to the young. Clinically, several investigators have reported the relative bacteriolytic values of the mother's and infant's sera at the time of birth (Halban and Landsteiner[46]), the infant's serum showing a less amount of natural bacteriolysins than the mother's serum.

b) Hemolysins. Several investigators have reported upon the transmission of specific hemolysins from immunized mothers to offspring either through the placenta or the milk. Immunized rabbits appear to transmit the hemolysins to the fetus through the placenta (Bulloch,[15] Bertino[50]). Goats may or may not (Kraus,[44] Kreidl and Mandl[51]). In one case sheep failed to do so (Bertarelli[48]). Natural hemolysins have been reported as being present in some cases in the serum of infants at birth. The question of the transmission of hemolysins will be taken up later in this paper, and be fully considered.

5. *Opsonins.*—Turton and Appleton[52] compared the opsonic index of mother's and infant's sera in two cases (infants' ages, four and seven days) and found the infants' sera much lower than that of the mothers. Also they found, in comparative tests between the mother's serum and milk, that the latter was very much weaker in opsonins. It was not known whether the infants absorbed opsonin from the milk or not. This work has been in the main supported by other observers, both in human cases and by animal experimentation (v. Eisler and Sohma[53]).

6. *Allergin.*—It has been shown that female guinea-pigs which have been sensitized by injections of proteins, as horse serum, bear young which are also hypersensitive to the same serum during the early weeks of life (Rosenau and Anderson,[54] Anderson,[55] Gay and Southard[56]).

B. Antibodies of Poorly Differentiated Types.

1. *Bacterial, intracellular toxins.*—As is well known, a large group of pathogenic bacteria do not excrete specific, soluble toxins to any extent, so their poisonous prop-

erties are supposed to be mostly due to cellular proteins or so-called intracellular toxins. Active immunity may be developed by recovery from the disease, or by experimental vaccination with killed or attenuated cultures. Not much is known concerning this type of immunity, but it is probably quite complex in nature and dependent upon a number of factors. However, it has been experimentally proved that a mother immunized with certain members of this group may transmit a specific immunity to her young; for example (1) anthrax (Chauveau[3]); (2) swine erysipelas (Ehrlich[6]); or (3) tuberculosis. Behring[57] observed that a 14-day-old calf, born from a highly immunized cow (bovine tuberculosis), possessed considerable immunity against the organism. Behring thought that this immunity was acquired by the calf through the milk rather than by intrauterine transmission. He suggested the milk of specifically immunized cows as a means of combating tuberculosis in man, either as a preventive or a therapeutic measure. (4) Dysentery. De Blasi[58] actively immunized a rabbit against the dysentery bacillus by three inoculations of killed cultures during the first 18 days after birth of young. The young (two) were given on the 19th day surely fatal doses of the dysentery culture. Both remained alive, whereas controls died.

2. *Spirilla.*—*a*) *Sp. obermeieri.* Animals may be actively immunized against this organism by further injections of spirillar blood following a recovery from the infection; others may be passively immunized by the injections of serum from such animals. Novy and Knapp[59] found young rats, born from infected mothers, immune when tested after a few weeks. They looked upon this as an active immunity, due to intrauterine infection, since the spirilla may penetrate the placenta and reach the fetus. Therefore it remains an open question if an active immunity (intrauterine infection), or passive immunity (placenta, milk) from mothers' antibodies occurs in the young.

b) *Spirochaeta pallida* (sỳphilis). It is questionable whether a complete immunity ever follows in syphilitic patients after a clinical recovery from the disease. With the introduction of the complement fixation test in the diagnosis of syphilis, it has been found that many apparently healthy children born from luetic mothers give a positive reaction, while others, nonsyphilitic, may contract the disease by suckling a diseased mother (Ricketts and Dick[60]). This would prove that Profeta's Law* can no longer hold. It would appear that whatever immunity exists is slight. According to Thomsen,[61] a number of observers besides himself have found complement fixing substances in the milk from luetic women. It would be of interest to know if an infant taking such milk from a luetic mother would absorb these bodies in sufficient quantity as to give a positive reacting serum. It is still questionable whether these complement fixing bodies are true antibodies, or simply metabolic products brought about by pathological changes in the body of the patient during the course of the disease.

3. *Protozoa.*—*a*) Piroplasmosis (dog). Kleine and Möller[62] showed that immunized dogs transmit some immunity to their young against this disease.

b) Rabies. This disease is only tentatively placed under this division since the question of the protozoan nature of the so-called Negri bodies is still under discussion. Hoegyes[63] was one of the first workers to point out that actively immunized female dogs may transmit some immunity to their offspring. Konradi[64] carried out similar experiments upon dogs and in several cases with positive results. The work of Remlinger[65] with rabbits also supported those results.

4. *Ultramicroscopic organisms.*—Under this heading may be placed a group of

* Profeta's Law assumed that a healthy child might be born from a luetic mother, and suckle the mother without contracting the disease.

contagious diseases which, on account of the constant failure to demonstrate definite specific microorganisms, has led many workers to consider the contagion in each case to be ultramicroscopic in size. (*a*) Sheep-pox (ovine). According to Burckhardt,[2] Richert immunized a large herd of sheep against ovine during the last six weeks of gestation. The lambs of these mothers were inoculated with ovine-lymph at the age of four to six weeks. None of them contracted the disease. A large number of controls showed pustules.

b) Foot and mouth disease. Loeffler[66] observed a calf born from a cow immunized against this disease, which proved to be immune against inoculations of the virus.

c) Small-pox and vaccinia. Since in the introductory portion of this paper small-pox has been briefly but sufficiently discussed, it will not be considered further. The work of Burckhardt[2] upon small-pox vaccine is of special interest since it was one of the first investigations upon the possible transfer of protective substances from mother to the child *in utero*. He vaccinated mothers during the last weeks of pregnancy, and then repeated the vaccinations upon the infants during the first days of their lives. He found in several that an apparent protection against the vaccinia had been acquired. Most of the control infants gave positive reactions. While these results were not conclusive, they offered suggestive data. In this connection it may prove of interest to note that in 1799, Hufeland (cited by Neumann[67]) was perhaps the first to suggest that the milk might exert a protective power for the child when secreted by a mother who had recently recovered from the disease. In this class of diseases, as has been previously pointed out, it is always a question whether the young have been passively immunized from the mother's antibodies, or if an active immunization has taken place from having had the disease before birth.

III. DISCUSSION OF RESULTS.

In reviewing the literature on this subject, one is impressed by the broad scope of the studies, and the great amount of work which has been done. Even in the brief review just given, it is evident that the work on active immunity has been very productive of positive results. Also much work has been done by passively immunizing pregnant and nursing animals, but this has been purposely omitted in our résumé, since our studies cover only actively immunized mothers as influencing the offspring.

The possibility of an active intrauterine immunization on the part of the fetus must always be considered in connection with this type of experiment. Such may occur either through the passage of the living infectious agent or its products from the mother to the fetus. A distinction must be made between this condition and a passive immunization by means of the mother's antibodies through the placenta or through the milk to the young. In the diseases which are supposed to be due to ultramicroscopic organisms, the virus not infrequently seems to be able to pass the placenta and

affect the fetus *in utero*. Where immune offspring are born from mothers which have passed through such a disease during pregnancy, caution must be exercised against accepting this as a true passive transmission of immunity (*in utero*) from the mother. To this we must add that animal experiments with certain living spirilla must be considered in the same light, since they may also pass the placenta and affect the fetus.

Most bacteria do not pass the intact placenta except under extraordinary conditions. Further, recent work (besides our own) would indicate that the fetus, or the very young animal, possesses only feeble reactive powers as to antibody production, when attempts have been made to actively immunize by means of prepared antigens. As shown by the foregoing review, fluids from fetuses aborted early in pregnancy by infected mothers usually gave negative results when tested for specific antibodies. Judging from these investigations we should expect the best results in the young from animals which have been actively immunized either against dead cells or toxins during the latter part of gestation. Even where such conditions were observed, positive results did not follow in every case. In part, the negative results may be accounted for by differences in experimental conditions, such as nature of antigen used, degree of active immunization attained, time of immunization relative to birth of young, character of resulting antibody, time when blood from offspring was first taken, etc. Further, many workers failed to make any distinction between the rôle played by placental or by milk transmission of antibodies; in many cases the blood of young was not drawn until after having the mother's colostrum and milk. In fact, blood samples in some instances were not taken until weeks after the birth of the young. Much of the literature cited in the above review covers very careful and painstaking investigations which have been carried out upon this subject. These studies prove that when the mother has been properly immunized during gestation and shows a high degree of immunity at the time of birth of young, some of this immunity (antibodies) may be passively transmitted to her young. This occurs either through the placenta before birth, or by means of the milk immediately after birth.

IV. REPORT UPON ADDITIONAL WORK CONCERNING TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING.

1. *Introductory remarks.* As pointed out in the foregoing résumé, the passive transmission of antibodies to the offspring is practically established. This seems to hold for almost all classes of infectious organisms where a true active immunity (formation of antibodies) takes place in the mother's body. But the relative importance of the exact parts played by the two separate means of transmission—placenta or milk—has not been so fully decided. Some workers maintain that the placenta plays the greater rôle, while another but smaller group holds that antibodies do not pass the intact placenta. More work upon this subject, bearing in mind this phase of the question, seems desirable.

A large group of infectious diseases are induced by organisms which do not produce extracellular toxins (in culture) sufficient in amount or of such a nature that antitoxins can be produced by animal immunization. Recognizing the importance of this group of endotoxin-producing organisms, and their significance in disease, it was thought worth while to take up analogous studies which might throw more light upon the transmission of immunity to offspring. Since if by vaccinating the mother cautiously during pregnancy, the offspring also becomes immunized, a means is offered by which the young may be protected against those infections (pneumococcus, etc.) to which they are particularly susceptible during the first weeks of life. Up to the present time, we have no curative serum which offers much hope in these conditions. Active immunization (by vaccines) of the newly born against these organisms offers little encouragement and, in fact, may be followed by harmful sequelae. Not much is known concerning the nature of the immunity induced by actively immunizing animals against this type of organism. But perhaps in so far as the serum of such animals is concerned, the contained antibodies are quite complex in nature, and include antitoxins, bacteriolysins, opsonins, etc. Also there seems to be evidence that such a serum contains substances of which we know little, other than that they produce favorable results, exclusive of the bodies already mentioned. Since cytolysis must be one of the important factors in this type of

immunity, the cytolysins may act as an index in carrying out experimental studies upon such immune sera.

As mentioned in the review, little has been done directly upon the bacteriolytic antibodies concerning their transmission from mother to offspring, but bacteriolysins have been found in milk from different animals. Most workers who have carried out studies along cytolytic lines have used lysins against red blood cells—the hemolysins.

In my own work, which is to follow, this means was adopted, as it appeared most advantageous. For instance, the test can be carried out completely in test tubes, and, under proper technic, with a high degree of accuracy. Also, test animals, as necessary in diphtheria or tetanus antitoxin work, can be entirely eliminated. As compared with bacteriolytic work the hemolytic tests are much more rapidly performed and probably more easily graded.

2. *Review of work reported upon transmission of hemolytic antibodies.*—After a careful search through the literature I find that several investigators have already reported work concerning the transmission of hemolysins from mother to offspring. However, some of this work escaped my attention until after my own work was well under way. Although briefly mentioned in the previous general review, these reports will now be considered more fully.

Kraus,[44] in 1901, reported his work upon the possible transmission of immune hemagglutinins and immune hemolysins from mother to offspring. He found that rabbits immunized by repeated subcutaneous injections of defibrinated dog's blood showed in their milk specific hemagglutinins, but these bodies were not carried over to the blood of the suckling. For the study of hemolysins, he immunized goats by injecting with sheep blood. No hemolysin could be found in the milk, so he concluded that these immune bodies were not excreted by the mammary glands, although the blood serum of the same animal showed quite definite amounts of the hemolysin. However, he found that the young born from one of the immunized goats showed a low degree of immunity (hemolysin) which was transient. Kraus thought that this was acquired before birth, and considered it a passive immunization from an actively immunized mother. He gives no definite data concerning the hemolytic tests of the serum but apparently he added no normal complementing serum, but depended upon the small amount of complement which was present in the immune serum used in the test. As is well known, goat serum is weak in hemolytic complement, and therefore will not bring out the highest efficiency of the contained immune hemolysin, even when present in large amounts. As a result, his data show the hemolytic sera to be of very low value. But in the milk tests he added normal goat serum, as complement, since he thought it possible that the milk glands might not let the complement through

consequently no activation of the immune body could take place in case it were present. In view of negative results, he concluded that immune body was not present in the milk. He did other experiments with goats' milk which indicated that it was deficient in complement.

Following this, Bulloch,[15] in 1902, took up the study of transmission of hemolysin from parent to offspring, using rabbits immunized against blood cells of the ox. He considered not only the rôle of the immunized mother, but also that of the father from the standpoint of heredity, and obtained results which, on the whole, supported the early work of Ehrlich. But of special interest to us are his findings concerning the transmission of specific hemolysin from mother to the young. When the blood injections were given to the gravid animals, pregnancy was interrupted, but the fetal fluids were found strongly hemolytic. In those cases in which the injections were made prior to conception, the young were reared, and in one case the inactivated serum of the young was equal to that of the mother in hemolytic power, while the milk was only one-third as active as the mother's serum. In another case the serum of the young, which was found dead on the day of birth, was one-half the value of that of the mother, while the milk was about one-fourth of that value. Bulloch agreed with Kraus that no complement passed out with the milk,* but showed in opposition to Kraus that hemolytic immune body is excreted by the milk glands. He used fresh guinea-pig serum as complement in all of his tests.

Kreidl and Mandl[51] (1904), in a preliminary report, stated that goats injected with beef blood might lead to a passive immunization of the fetus, but from their tests concluded that in the majority of cases, the specific hemolysins do not pass from mother to fetus through the placenta. They considered individual variations to be responsible for the lack of uniformity in their results. No definite experimental data are given in this report. They also consider the transmission of immune hemolysins from the fetus over to the mother, but as their results are not fully conclusive, they will not be discussed in this connection.

Bertino[50] (1905) states that in rabbits, when the immunization of mother has taken place before conception, the lysins do not pass over to the fetus, nor to young during the suckling period, but when the immunization is carried out during pregnancy, there is a transmission of the specific antibodies to the fetus. Since the original article was not available these results are quoted from an abstract (*Folia Haematologica*, 1905, 2, p. 624), and I am unable to give further details.

Bertarelli[48] (1906) reports that he found a small amount of specific hemolysins in the milk of a sheep immunized against hen's blood, but found none in the blood of the young. This immunization was carried out by repeated injections of the blood during the last weeks of pregnancy and the first weeks after birth of lambs.

Considering the report just presented, it would appear that hemolysins may be transmitted through the placenta to the fetus *in utero*, especially in the examples cited by Bulloch. But objections may be raised as to the validity of the findings from aborted fetuses, since in those cases a placental lesion could exist which

* Lane-Claypon (*Jour. Path. and Bact.*, 1908–9, 13, p. 34) claims to have demonstrated complement in fresh milk (in about 1/10 value of the individual's serum) as well as natural immune bodies, by the addition of the "ox colloid" of Bordet. Others have also shown the presence of the hemolytic complement in milk (Pfaundler and Moro; *Ztschr. f. exper. Path. u. Therap.*, 1907, 4, p. 451).

would permit a direct connection between the maternal and fetal circulation. Apparently the ox blood injected seemed to exert a toxic action upon rabbits. It would be difficult to exclude the influence of absorption of hemolysins from milk previous to time blood samples were taken from young, in some of the cases. The question of animal species may enter largely in determining the results. The meager data available in Bertino's work upon rabbits would support the statement of Bulloch. Perhaps larger animals, as goats or sheep, do not permit this class of antibodies to pass the placenta so readily as some of the smaller animals.

Another important point, which must be considered in such experiments involving specific hemolysins, is the presence of natural hemolysins (for the blood used) in the blood serum of the mother, and their development in the body of the fetus, or possible passage over from the mother's circulation. Much variation in this respect may exist between different individuals of the same species. It is necessary carefully to control adult serum as to content of natural immune bodies against the particular blood to be used for immunizing purposes, before any injections are made.

3. *Choice of experimental animals and outline of technic.*—In the present work goats were selected for experimental animals since they are more highly developed, rugged, and withstand experimental handling (injections, bleedings, etc.) very readily, and usually give a bountiful supply of milk. Sheep corpuscles were used for immunizing purposes; their advantage being that goat serum seldom shows the presence of more than a small content of natural hemolysins, if any, against these corpuscles. Goats readily respond to the blood injections by specific hemolysin production. This immunity may be raised to a high degree by repeated injections of the blood, while practically no toxic action is exerted upon the animal. Moreover, only a small amount of hemagglutinins are formed by goats against sheep corpuscles; hemagglutinins when present in large amounts, in a hemolytic serum, cause a marked disturbance in the hemolytic reaction.

In my experiments, the technic followed was similar in all cases, and may be briefly outlined as follows:

1. Preparation of sheep's blood for injection, or for hemolytic test: Blood was drawn from the jugular vein of normal sheep by puncturing the skin and vessel wall

with a large hollow needle and collecting the flow in a sterile bottle containing a coil of wire. It was defibrinated by shaking, then placed in tubes, centrifuged, the serum removed, and then the corpuscles were washed three to four times in 0.9 per cent NaCl solution until they were practically free from all serum.

2. The injections of blood in these experiments were all made subcutaneously. If the volume was very large, it was divided and injected into the animal in several places.

3. Collections of blood samples. Bleedings were made from the jugular vein. In case of adult, a hollow needle was used, but from kids, a few c.c. were drawn by means of a hypodermic syringe. In each case blood was placed in a test tube and allowed to clot out. When the serum had separated, it was removed and placed in small sterile bottles.

4. Milk samples had 10 per cent by volume of 1:1,000 formaldehyde in 0.9 per cent NaCl solution added. Preliminary tests showed that this strength of formaldehyde solution exerted an antiseptic action, while it had little if any influence upon hemolytic antibodies in milk. The milk samples were then placed in bottles for storage. Both serum and milk samples were stored in ice-box where they were kept until the entire series covering the experiment were collected. Tests were done with the fluid portion which was removed from under the cream layer, after this had separated. The first colostrum, which was heavy and tenacious, failed to separate a cream layer, so was used as a whole.

5. When a full series of samples of sera and milks for a given experiment were collected, all were tested at the same time, thereby using the same blood suspension, complement, technic, etc., which made the results much more comparable. Preliminary tests were first made to get relative values of the samples. The serum became "inactivated" by aging; no heat was applied in any case. The ordinary test tube method for the determination of specific hemolytic immune body* was employed.

For complement, in the earlier experiments, fresh rabbit serum (0.20 c.c. per tube) was used; later, fresh serum from guinea-pigs (0.10 c.c. per tube) was used. This proved to be more efficient, and was used in all tests which are cited except when otherwise noted.

The scheme followed for the tests was to arrange a series of test tubes for each sample; the serum or milk which was to be tested was first accurately measured in decreasing amounts directly from sample or from the proper dilution in physiological (0.9 per cent) salt solution dilution, into a series of test tubes. Then physiological salt solution was added to bring the volume of each tube up to 1.0 c.c. To this was added the given volume of complementing serum, then 2 c.c. of 7.5 per cent suspension of sheep corpuscles. Immediately the tubes with the mixtures were thoroughly shaken, then placed in the incubator (36°–37° C.). After one hour they were removed, again thoroughly shaken and placed in the ice-box until any remaining, intact corpuscles settled to the bottom of the test tube. After the corpuscles had sedimented, a transparent fluid remained above, varying from a deep red color to a water white, depending upon the presence or absence of hemolysis. The results of the tests were read by making equi-colorimetric readings throughout the various series of samples by com-

*The term "hemolytic immune body" is used in this report as a more suitable equivalent than "substance sensibilisatrice" (Bordet), or "intermediary body" "amboceptor" (Ehrlich) for the biological reaction product of the organism against injected alien blood cells.

paring with a "standard color" tube which was made by "laking" a given volume of the blood corpuscles in distilled water.

4. *Groups of experiments.*—For convenience the experiments will be considered under four groups; the first two cover the main tests upon the transmission of hemolysins from the mother to the young, while the remaining groups cover certain tests which have a general bearing upon the subject.

In the first group the female goats were actively immunized against sheep corpuscles by the method outlined. Each experiment was a comparison of the relative content of specific hemolysins in the mother's serum, the serum of her own young, and of her colostrum or milk. No "cross nursing or feeding" of young was undertaken. The tests are not given in perfect chronological order, but each experiment will be given a number for reference purposes, and these will run consecutively throughout the report. In order to avoid repetition, typical experiments will be reported in full; others falling under the same head will be briefly described.

a) Active immunization of the adult before birth of the young, either during the period of gestation, or before conception had taken place.

SERIES 1. PRELIMINARY EXPERIMENTS.

To obtain data concerning the possible transmission of hemolysins from mother goats to their young, preliminary experiments were undertaken. Since the results proved instructive from several standpoints, a part of the data will be submitted. In this series, pregnant animals were immunized during the last weeks of gestation or immediately before the birth of the young. Blood samples were taken from the adults before the injections were started, and then at short intervals during the course of immunization. On day of birth of the young, blood samples from the mother and young, and a sample of the mother's colostrum were taken. Following this, samples were taken at intervals for two and a half to five weeks or even longer.

Experiment 1. Goat 4 (normal; pregnant).—During the course of nine days this animal received six subcutaneous injections (increasing doses) of washed sheep corpuscles—a total of about 400 c.c. On the morning of the 12th day after the last injection it was found that she had given birth to two normal kids; one was removed from the mother about noon, and placed on cow's milk although it had undoubtedly

freely suckled its mother. Late in the afternoon blood samples were taken from each kid. During the same forenoon, both blood and colostrum-milk samples had been taken from the mother. Test samples from each were taken at intervals for over two weeks following the birth of the young. Comparative tests (regular technic, 0.20 c.c. rabbit serum for complement) were made upon all of the samples at the same time and the following results were given:

Time	Mother's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young	0.00 c.c.*	0.02–0.03 c.c.	0.035–0.04 c.c.	0.045 c.c.
1st day after birth		0.20–0.30 "	0.04 "	0.05 "
2d " " "	0.055 "	Traces	0.045 "	0.08 "
4th " " "	0.00 "	0	0.06 "	0.08 "
6th " " "	0.035 "	0	0.08 "	0.09 "
10th " " "	0.047 "	0.20 c.c.	0.10 "	0.175 "
14th " " "	0.075 "	0	0.15 "	0.20 "

*When comparisons are made between the samples it must be understood that the potency value is inverse to the quantity value. The numbers indicate the volume of each sample which was required to produce a given degree (constant) hemolysis in that test.

Further tests showed that the antibody content of the blood in both kids continued to fall, more or less in parallel. The adult's serum showed its highest value on the sixth day after the birth of the young (18th day after the last injection of blood), then gradually declined. The milk showed transiently (eighth to 10th day) demonstrable amounts of antibodies, following shortly after the adult serum showed its greatest concentration in antibodies.

Experiment 2. Goat 14 (normal; pregnant).—Two subcutaneous injections of washed sheep corpuscles were given on consecutive days: first day, 125 c.c.; second day, about 140 c.c. Twenty-one days after the last injection, the mother was found in the morning with one normal kid which was allowed to remain with her. At noon blood samples were taken from both and also a sample of the mother's colostrum-milk. The kid had suckled and stomach seemed full at time the bleeding was made. During the course of immunization, blood samples were collected from the mother, and this was continued with the addition of milk samples at intervals for some time after parturition. Likewise, blood samples were taken from the kid, and finally all samples were tested at the same time to get the relative hemolytic values. The adult's serum showed its maximum hemolytic value (0.0075 c.c.) 13 days before birth of young. From this high value it rapidly receded until on the day of birth of young it gave a comparative value of only 0.035 c.c. It remained fairly constant at the same level for about one week, then gradually fell. The colostrum-milk showed a value of 0.05–0.06 c.c. on day of birth of young; the antibodies rapidly disappeared from the milk so that by the second day after the birth the test failed to show their presence. The kid's serum from blood samples taken on day of birth showed a value of 0.017 c.c., while a sample taken a few days later showed about twice this value, then gradually receded. This would lead us to believe that gastro-intestinal absorption of hemolysins from the milk is an important factor in the very young animal. The first blood sample was taken from the kid a few hours after birth and although it had freely fed, the absorption of antibodies was still incomplete but the assimilation from the digestive tract continued after this time, as was indicated by higher value of its blood serum taken later. This question will be taken up again and more fully discussed.

Experiment 3. Goat 11 (normal; pregnant).—This animal was given subcutaneous injections of washed sheep corpuscles spaced three days apart—three in all—100 c.c., 75 c.c., and 75 c.c. A kid was born during the evening or night following the last injection; it was allowed to remain with the mother. The mother's serum showed no definite immune bodies on day of birth of the kid. Two days later blood samples from both were drawn and a milk sample was taken from the mother; this was repeated on alternate days for several weeks. All were tested at the same time, using 0.20 c.c. of rabbit's serum for complement. The mother's serum showed its highest value in specific hemolysins on the 10th day after last injection of blood cells, then gradually receded from this point. The milk showed a low antibody content; the mother's serum and milk reached their highest concentration simultaneously (serum 0.0035 c.c., milk 0.20–0.25 c.c.). The kid's serum showed questionable traces of specific hemolysin on the fourth and sixth days after birth but none later, although the blood serum was watched for several weeks. This test shows that an immunity to erythrocytes arising during the period of lactation has an influence upon the milk, but that the antibody content is less than 2 per cent of that of blood. Further, a kid at this age must receive much higher quantities of antibodies through the milk, in order to absorb a sufficient quantity to be easily demonstrable in its blood serum. This will be more fully supported by results of other tests which are to follow.

These preliminary tests indicate that mothers actively immunized against the erythrocytes during the latter part of pregnancy can transmit these antibodies to the offspring. But when the immunization has progressed only slightly, that is, no antibodies are demonstrable in the blood until after the birth of the young, the young fail to show more than a trace of antibodies in their blood. Presumably these are acquired through the milk. Probably in all cases this transmission was passive, and took place either through the placenta or the colostrum-milk, or both. I was led to think from these early tests that of the two factors each was about equal in importance. It was found that the colostrum-milk was rich in specific antibodies when the females were immunized before parturition. Also, the kids showed a rise of antibody content in their blood, as they took and absorbed this colostrum, which rapidly reached a maximum and then decreased slowly, as in passive immunization. Further, it was observed that an animal which received first blood injection only a few days before and the last on day of birth of young, did not influence the offspring to any appreciable extent; the milk showed a very low antibody content.

SERIES 2. SPECIAL EXPERIMENTS.

A series of experiments were undertaken by which the relative importance of the two factors—placenta and colostrum-milk—in

the transmission of hemolysins to the young could be determined. For this purpose goats were actively immunized against sheep erythrocytes in the regular manner either during the course of gestation, or before conception had taken place. Blood samples were taken from each goat before the injections were started, and at short intervals during the course of immunization. To exclude the possibility of the young getting any milk immediately following birth, the ends of the mother's nipples were previously sealed with collodion. After birth of the young, blood samples were taken; the collodion seals were removed from the mother's nipples, a colostrum sample taken, then the newly born animal, which was assigned to the mother, was allowed to suckle. In case twins came, one was never allowed to suckle the mother but was put directly upon cow's milk as a control. Test samples were taken from mother and young at frequent intervals for some time following the birth. In all tests in this series 0.10 c.c. fresh guinea-pig serum (per tube) was used as complement.

Experiment 4. Goat 29 (normal; pregnant).—Six subcutaneous injections of sheep erythrocytes were spaced over 16 days in gradually increased doses, a total of 450 c.c. blood cells. On the 34th day after the last injection, the animal gave birth to two kids. Blood samples were taken from both before they were allowed any milk. One was removed and placed upon cow's milk, while the other was allowed to take mother's colostrum after the collodion seals were removed and a sample drawn. The serum from a blood sample taken from the mother before first injection of sheep blood, showed a definite amount of normal hemolysin (0.10 c.c.) against the sheep corpuscles used in the test. During the course of the immunization the mother's serum showed the highest antibody concentration (0.008 c.c.) nine days after the last injection; the content rapidly fell so that on day of birth of young (34th day after last injection) it was quite low. Since 0.10 c.c. of the mother's serum showed a definite amount of natural hemolysins against the sheep corpuscles, no greater volume of the test samples were used. The results of the comparative tests are as follows:

Time	Adult's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young.....	0.06 c.c.	0.018 c.c.	0	0
1st day *after* birth of young..	.....	0.08 "	0.048 c.c.	0
2d " " " " " ..	0.070 "	?	0.045 "	0
4th " " " " " ..	.....	0	0.050 "	0
7th " " " " " ..	.....	0	0.058 "	0
10th " " " " " ..	0.10 "	0	0.060 "	0
13th " " " " " ..	Trace	0	0.060 "	0
17th " " " " " ..	Trace	0	0.075 "	0

It is shown by the above data that the adult rapidly lost its specific hemolysins—even the natural hemolysin seemed to have diminished. According to the test, neither of the kids showed any trace of hemolysin in 0.10 c.c. of their sera taken on

the day of birth; the one on cow's milk continued negative, while the one with the mother acquired, by absorption from the colostrum-milk, a definite hemolytic content in its blood. The colostrum showed a relatively high amount of hemolysin at time of birth of young, but this rapidly diminished until the test failed to show its presence.

Experiment 5. Goat 31 (normal; pregnant).—Received every third day an injection of sheep corpuscles until six were given—total volume injected was 390 c.c. Samples were taken both before blood injections were made and at intervals during the immunization. Six days after the last injection, the animal was found with two normal young. Since the mother's nipples were sealed, neither kid had any milk; blood samples were taken from each, then one was removed and placed on cow's milk; the other was allowed to suckle mother after the collodion seal was removed and a sample of colostrum was taken. Unfortunately this experiment was of short duration, since the kid with mother became ill (probably from cold) the second day after birth, and experiment had to be discontinued. However, the results are of sufficient interest to be briefly tabulated here.

Time	Mother's Serum	Colostrum-Milk	Kid "A" Serum (Mother's Colostrum)	Kid "B" Serum (Cow's Milk)
Day of birth of young.....	0.0075 c.c.	0.015–0.020 c.c.	0	0
1st day after birth.....	.....	0.035–0.040 "	0.05 c.c.	0
2d " " "	0.0097 "	Trace	.....	0

This test shows that while both blood serum and colostrum from mother contains a high antibody content, neither kid had sufficient antibody in blood at birth to give even a trace of hemolysin when 0.10 c.c. serum was used, but the one which had taken mother's colostrum on day of birth, showed a fairly high amount of the specific antibodies in its serum on the following day.

Experiment 6. Goat 15 (pregnant).—This animal had been immunized (sheep erythrocytes) five months previously, but at the time this experiment was undertaken no specific hemolysins were demonstrable in the blood serum. Again subcutaneous injections of sheep blood cells in gradually increasing doses (six) were given, three to four days apart, making a total of 250 c.c. in all. A test showed that the serum reached its highest value (0.001 c.c.) on the seventh day after the last injection, but at the time of birth of kid it had greatly decreased (0.020–0.025 c.c.). One normal kid was born 115 days after the last injection. Shortly after birth a blood sample was taken from the kid; the seals were removed from the mother's nipples and a sample of colostrum was drawn. Then the kid was allowed to remain with the mother and suckle. On testing the samples the mother's serum showed a relatively low content (0.020–0.025 c.c.) of hemolysin, which remained quite constant for some days after the birth of young. The colostrum while scant was especially high in its hemolytic content (0.009 c.c.) on day of birth. On the following day the milk showed a very low test (0.15 c.c.) and was entirely negative by the end of the week. A sample of 0.30 c.c. of the kid's serum at birth did not show a trace of hemolysin by test, so probably little if any passed the placenta from the mother. On the second day after having suckled, only a trace of hemolysins could be demonstrated in the kid's serum; this can be explained in part by the known fact that the mother had only a small amount of colostrum, the volume of milk secreted was scant for several days, and the kid showed signs of hunger and under-nourishment.

Experiment 7. Goat 6 (pregnant). It may be of interest to record in this connection an observation made on another goat which might be classed with this series. The mother showed signs of oncoming labor during the late afternoon, so both blood and colostrum samples were taken. Next morning it was found that the animal had given birth to two large (weight four lbs. each), apparently fully developed young which were dead. Nothing in particular was shown on autopsy excepting some subcutaneous edema in each; some bloody fluid was found, in the thoracic cavity, which was removed and saved for test; the heart and large vessels were fairly well filled with partially clotted blood which was removed and the serum collected; lungs, nondilated, on section showed nothing unusual; the other organs appeared normal. Possibly a large bleeding (between 300–400 c.c.) which was taken from the mother one week before, might have had some influence upon the welfare of the young. The weight of the adult, on day following the birth of young, was 83 lbs. Upon centrifuging the fluid from the thorax, and the blood from heart and great vessels, it was found that in one kid the fluids came out quite clear, while those from the other were rather deeply colored by the presence of hemoglobin. We shall therefore consider the tests made upon the clearer fluids from the one kid. The following comparative values were shown: Mother's serum, 0.0025 c.c.; Colostrum, 0.0015–0.0020 c.c.; Kid—the fluids from the blood and from the thorax failed to show a definite hemolysis in amounts up to 0.20 c.c.; a very slight tint was observed but it is questionable if this were due to specific antibodies or traces of hemoglobin in the sample.

This adult was unique in the sense that she seemed to retain a very high antibody content in her blood long after the last blood cell injection. The last blood injection was given about six months (170 days) before birth of the young. Most animals lose their antibodies much more quickly; few show them in appreciable quantities after six months. This animal was immunized before conception had taken place, since the period of gestation in goats is stated to be five months. If the hemolytic immune bodies passed through the placenta to the fetus in this case, the amount was very small.

From the experimental evidence given in this last series of experiments it would appear that the colostrum plays a greater rôle in the transmission of hemolytic immunity to the offspring than the placenta—the latter probably transmits little if any appreciable amount of hemolytic immune body, to the fetus *in utero*.

b) Active immunization of the adult following immediately or shortly after birth of young.—A number of workers have succeeded in highly immunizing female animals, during the period of lactation, against toxins and getting the antibody in the milk in a relatively high concentration. Since it has been proved that the milk is an important factor in transmitting antibodies to the suckling during the first days of life through gastro-intestinal absorption, it was thought worth while to undertake similar experiments at later periods in connection with our studies.

SERIES 3. ANIMALS NORMAL AT TIME OF PARTURITION.

Experiment 8. Goat 8 (normal). Preliminary test.—This animal gave birth to one normal kid which was allowed to remain with and suckle mother. Blood samples were taken from the mother and kid, then the mother received a subcutaneous injection of 75 c.c. of sheep corpuscles. Following this, 100 c.c. corpuscles were given daily for three consecutive days—a total of 375 c.c. Blood samples were drawn from the mother and kid on alternate days for two weeks, after which samples were taken every third day for some time longer. In this experiment no milk samples were taken during the first weeks as the supply was scant; the serum samples were tested at the same time using 0.20 c.c. rabbit serum per tube for complement. It was found that antibodies did not appear in definite amounts in the mother's serum until five days after the last blood injection, then they rapidly increased until the 11th day, when the highest concentration (0.0035 c.c.) was found; the antibodies slowly declined until on the 46th day after last injection the value had fallen to 0.037 c.c. Serum samples from the kid when tested in amounts as high as 0.5 c.c. failed to show positive results. It is questionable if any appreciable amount of hemolysin was excreted in the milk when the mother's serum was at its greatest concentration. If so, the kid at that age failed to absorb (unchanged) a sufficient quantity to give a definite hemolytic reaction in the test.

Experiments 9 and 10.—Similar experiments were performed with goats 4 and 5, with the exception that milk samples were taken throughout and tested at the same time as the blood serum samples of the respective mother and kid. In neither case did we succeed in getting very high immunity in the mother animals, and it was found that an appreciable amount of antibody failed to pass over in the milk. Naturally the serum of the kids did not show antibodies.

These experiments proved quite unsatisfactory. It appears that an exceedingly high immunization of the female is necessary before a fairly high antibody content occurs in the milk. In neither of the two goats last mentioned was the degree of immunization sufficient to cause a demonstrable overflow of antibodies in their milk.

An obvious objection to the active immunization of normal females immediately after birth of young is the necessary delay before the production of antibodies in high concentration in the mother's body, and their appearance in the milk. In the meanwhile the young animal becomes more mature; its digestive powers are more pronounced, and probably anatomical changes occur in the gastro-intestinal mucosa which retard or prevent the absorption of the unchanged antibodies.

SERIES 4. ANIMALS PARTLY IMMUNIZED AT TIME OF PARTURITION.

In order partly to overcome certain objections to which attention has just been directed, animals which had been previously

immunized and still showed a low antibody content in their blood at the time of birth of the young, were reimmunized by more blood injections following parturition. In this way it was hoped that a high immunity could be induced in the mother, and more quickly than was possible in the strictly normal animals, and that the milk would either remain high or increase in immune body value. Possibly kids receiving such milk in quantity during the first weeks of life would be able to absorb the immune body unchanged, and as a result their sera would remain about constant or even show some decided rise in the hemolysins. In opposition, other factors enter, such as (*a*) animal growth (increase in body fluid), (*b*) loss through blood samples withdrawn, and the (*c*) metabolic destruction of antibodies. Therefore, such experiments should be well controlled.

Experiment 11. Goat 6 (previously immunized).—Found in the forenoon with normal twins, and as they had suckled, only a small sample of milk could be obtained from the mother. Both remained with mother until late in the afternoon. The mother was given a large injection of washed sheep corpuscles the same evening.* The blood injections were repeated every second day until four in all were given—a total of 425 c.c. Blood samples (adult and kid) and milk were taken for several weeks. All were tested simultaneously. The results of the test may be tabulated as follows:

Time	Mother's Serum	Milk	Kid "A" Serum (Mother's Milk)	Kid "B" Serum (Cow's Milk)
Day of birth of young	0.0080 c.c.	0.05–0.06 c.c.	0.002 c.c.	0.0035 c.c.
2d day after birth of young	0.0077 "	0.27 c.c.	0.002 "	0.0030 "
4th " " " " "	0.0040 "	0.16 "	0.003 "	0.0035 "
6th " " " " "	0.0020 "	0.08 "	0.003 "	0.0040 "
10th " " " " "	**0.0009** "	0.05 "	0.004 "	0.005 "
12th " " " " "	0.00098 "	**0.045** "	0.0042 "	0.005 "
16th " " " " "	0.0012 "	0.05 "	0.006 "	0.007 "
22d " " " " "	0.0017 "	0.15+ "	0.0078 "	..... "
28th " " " " "	0.0022 "	0.15+ "	0.009 "	0.019 "
34th " " " " "	0.0027 "	0.18 "	0.015 "	0.03 "
40th " " " " "	0.003 "	0.27 "	0.022 "	**0.045** "

(When 45 days old, Kid "A" weighed 14½ lbs., and Kid "B," 17 lbs.)

As evident from the above data, the kid which was with the mother and getting a bountiful supply of milk containing large amounts of immune body, showed in its blood serum an antibody content practically running parallel with that of a control (brother) which was fed upon cow's milk after the first day following birth.

* At the same time this animal received injections of killed cultures of the colon bacillus in small but increasing doses on days alternating with the blood injections. These injections were continued over two weeks without any harmful effects in so far as noted.

The ratio of weight between the two kids probably ran roughly parallel throughout the test. So it would appear, at least in this instance, that not many of the antibodies pass over unchanged to the circulation from the gastro-intestinal tract of a suckling animal after the first days following birth.

This experiment would suggest that even when we can get an animal highly immunized and the milk shows the presence of considerable antibodies, hardly an appreciable amount is absorbed unchanged from the digestive tract by the suckling after the first days of life. These results were supported by other observations, but in experiments where a control twin was not available.

c) Active immunization of newly born or older kids against foreign blood cells.

SERIES 5. BLOOD INJECTIONS INTO KIDS.

In connection with our studies where mothers received very large injections of blood during gestation, especially in the very last weeks, we must consider the possibility of soluble antigens passing the placenta to the fetus. Bang and Forssman[68] state that ox corpuscles gave an ether soluble fraction which possessed antigenic properties when injected into suitable animals. It is possible, therefore, that in the body also similar soluble products are liberated in quantity and eventually reach the blood stream from the large subcutaneous blood injections. Hektoen and Carlson[69] have shown by transfusion experiments with dogs, which had been previously injected (intravenous) with small amounts of goat's blood cells, that after several hours the antigen is fixed in the body of the injected animal, and not transmitted in sufficient quantity to the normal recipient to cause specific antibody production in the latter. But when large blood injections were given to a donor, then later a direct transfusion was made to a normal animal, the recipient as well as donor produced antibodies in due course of time. This showed that the body's ability to fix the formed antigen was limited. Therefore the possibility exists that, from large subcutaneous blood injections into gravid goats, soluble antigen might eventually reach the fetus. The question arises, If the fetus receives antigen shortly before birth, has it the power to react and

form antibodies, or may it retain the antigen and form antibodies shortly after birth? Any attempt directly to inject the fetus* *in utero* is necessarily surrounded by numerous technical difficulties. Moreover, the results thus derived would be justly subject to criticism. A few experiments were carried out on kids at different periods after their birth to test their reactive ability against sheep erythrocytes. The results may throw some light upon this side of the immunity problem.

Experiment 12. Kid 96 B (newly born).—This kid was a twin born from a mother which had been immunized against sheep corpuscles, over five months before parturition. The mother's blood taken six days before birth of the young showed a trace of hemolytic immune body in 0.10 c.c. serum, while a sample taken on the day of birth failed to show any hemolysin in like amount of serum. The colostrum on day of birth of young showed a small amount of immune body in 0.10 c.c., but the milk on the following day was negative. A blood sample was taken from the kid before it was allowed to suckle, then it was placed with the mother. The same evening 12.5 c.c. of sheep corpuscles were injected into the kid subcutaneously and the next afternoon another injection of the same amount of corpuscles was given. Blood samples (eight) were taken at intervals during the 15 days following the last injection. On testing it was found that 0.10 c.c. serum from blood samples taken the second and third days after birth showed a small amount of hemolysin, but this practically disappeared and soon showed only as a trace. Since the kid's blood sample taken before suckling showed no hemolytic action in the same amount, one must look to the colostrum as a source of its antibody on second and third days. The tests of samples, covering two weeks following last blood injection, failed to show more than a trace of hemolysis at any time, but perhaps this trace was slightly greater at the end of the second week. Adults usually show specific hemolysin production by the end of the first week after injection, and often this is at a maximum on the ninth to 12th day. We must conclude that the young in this case produced hemolysins only to a very slight extent (minute quantity) in response to the injection of sheep blood corpuscles in relatively large amounts.

Experiment 13. Kid 15 B (10 days old).—This kid was also born from an immunized mother, but failed to show any appreciable amount of specific hemolysins in its blood serum at the end of the first week after birth. On the 10th day after its birth the kid was given a subcutaneous injection of 10 c.c. of sheep blood corpuscles and an equal dose was given in the same way on the 12th day. Samples were collected at intervals for three weeks after the last injection. By the test,† all were negative (0.10 c.c.) until the ninth day, when a slight trace of hemolysin was shown; on the 12th day this was somewhat increased, a definite but slight hemolysis; 16th day, questionable trace; 20th day, 0.10 c.c. gave negative results. This would indicate that a slight but transient immunity reaction took place against the sheep blood cells.

* Kreidl and Mandl (*loc. cit.*) attempted injection of the fetus (goats) *in utero*, but were unsuccessful in the outcome in practically every case.

† Used the regular technic excepting 2.0 c.c. of a 5 per cent instead of a 7.5 per cent suspension of sheep erythrocytes were added per tube.

Experiment 14. 68 B (18 days old).—The mother of this kid had been immunized against sheep blood cells, some time previously. It was removed from the mother on day of birth and fed during the first days upon goat's milk and then later upon cow's milk. On the 18th day after its birth, 20 c.c. sheep blood cells were injected into it subcutaneously and two days later an equal volume was given in the same way. Blood samples were taken during three weeks following the last injection. Testing by the regular technic, it was found that 0.10 c.c. of serum showed a trace of antibody, on the 18th day after birth (first injection of blood). Probably it got colostrum, which contained immune body, on day of birth. These antibodies quickly disappeared and further samples were negative in amount tested, until six days after last blood injection, when a slight trace again appeared and gradually increased so that on the 12th day after the last injection of blood, a slight but definite hemolysis was produced by 0.10 c.c. of serum; this had again practically disappeared within a week. This experiment indicates that only a very slight immunity reaction occurs in a kid after it has reached the age of 18 to 20 days.

If we are permitted to draw any conclusions from these few experiments we should say that the newly born animal has only slight ability to form hemolysins during the first days following birth. Consequently we cannot assume that the fetus *in utero* has greater power, even if antigen should reach it through the placenta. But, on the other hand, it is more probable that the fetus has even lesser power to form immunity reaction product than the newly born. This seems to be a property which is only gradually acquired by the growing young, as they adjust themselves to their environment in the external world. The work of several investigators (Schkarin,[70] Moll[71]) would seem to substantiate the view that the organism of very young infants or animals have only a slight ability for antibody production.

d) Gastro-intestinal absorption of antibodies.

SERIES 6. FEEDING OF HEMOLYTIC SERUM TO NEWLY BORN KIDS.

As apparent from the data submitted, the newly born kid readily absorbs in an unchanged condition the specific hemolytic immune body from colostrum through the gastro-intestinal tract. These immune bodies do not appear in the kid's blood serum unless present in considerable amounts in the ingested colostrum. However, the amount in colostrum-milk may be comparatively low and still cause a decided increase in hemolytic value of the suckling's blood, if the total volume taken is large. The question suggested itself, Was this free absorption of immune body taken through the milk-colostrum peculiar to the combination? Or, are the immune

bodies readily absorbed by the newly born kid, when fed hemolytic serum, irrespective of the nature of the medium in which they are present? As we had an opportunity, this question was put to test in two cases by feeding newly born kids with homologous serum (goat) which contained specific hemolysins. The kids were taken away from the mothers immediately after birth and were never allowed to suckle. Owing to the lack of animals at the time this investigation was in progress, exact quantitative experiments upon this question, unfortunately, could not be continued. The following results may prove to be of some interest in that direction:

Experiment 15. Kid 112 B (newly born, weight 1,350 gms.).—A twin, which was not allowed to suckle mother, was removed immediately after birth and a blood sample was drawn. An immunized goat (15) was bled at once, the blood defibrinated and centrifuged to remove all corpuscles. About 30 c.c. of this supernatant serum was fed to the kid and no other feeding was made until the next day, when it was given cow's milk, which was continued from that time on. Unfortunately on testing this serum it was found to be of much lower value than suspected; test showed that 0.02 c.c. produced, however, a well marked hemolysis when tested by the regular technic, with the exception that 2.0 c.c. of 5 per cent instead of 7.5 per cent sheep blood suspension was used.

Samples of blood were taken upon four consecutive days after the feeding of the immune serum. When these samples (including the sample taken from the kid before feeding) were tested, in the manner just mentioned, none showed hemolysis in amounts of 0.15 0.20 c.c. If one-third of the antibodies were absorbed (unchanged) one would expect the test to give at least a slightly positive reaction in the volume of kid's serum which was tested. It follows that the absorption of unchanged antibodies from the digestive tract in this animal was not pronounced.

Experiment 16. Kid 97 B (newly born, weight 2,535 gms.).—Immediately after birth this kid was removed without being permitted to suckle. A blood sample was taken, then it was fed by pipette 15 c.c. of specific immune serum (goat 15) which had been kept under good condition, in freezing ice-box, for almost four months. This hemolytic serum, tested shortly after the feeding experiment, was of such potency that 0.002 c.c. almost completely hemolysed 3 c.c. of 5 per cent sheep blood suspension (0.10 c.c. guinea-pig serum for complement), while 0.001 c.c. gave a definite hemolysis. About one hour after this feeding the kid was given 150 c.c. of cow's milk. No further feeding was made until the next morning, when it was put upon cow's milk regularly. Blood samples were drawn daily at first, then at longer intervals, covering in all 15 days' observation. The tests were carried out upon these samples by the same technic (amount of blood samples, complement, and volume fluid) employed in the preceding experiment (Kid 112 B). It was found that the sample taken before the feeding of hemolytic serum was negative in 0.10 c.c.; the day following the immune serum feeding, 0.10 c.c. serum showed a well marked action; the second day after, the same amount of serum showed less hemolytic action; the hemolytic power gradually diminishing until, on the 13th and 15th days after the serum feeding, no action was shown by 0.10 c.c. This experiment showed that considerable absorption (approximately

10 per cent) of the unchanged hemolytic serum from the digestive tract took place, and appeared in the general circulation of the young animal. Recognizing the fact that no definite quantitative conclusions can be drawn from as few as two experiments, no more can be said than that these experiments suggest that antibody absorption while taking place is relatively meager when hemolytic sera are fed to newly born kids.

5. *General discussion of results.*—Before attempting to draw any definite conclusions from the above experimental data, it may be well to consider more fully some of the findings, and the related questions. In this brief discussion, not only our own results, but the correlated results of other workers, will be considered. The experiments clearly demonstrate that goats, when highly immunized against sheep erythrocytes during the latter part of gestation, transmit the specific antibodies to their sucklings. Apparently the placenta plays a very small part in this transmission, but the principal rôle is played by the antibodies which are present in the colostrum-milk and are readily absorbed from the digestive tract when taken by the newly born offspring. In those cases where the young born from immune mothers were prevented from taking the colostrum-milk until after a blood sample had been drawn, tests failed to show the presence of hemolysins in the kid's blood serum in sufficient amount to be appreciable by the method used. So we must conclude that the fetus *in utero* acquires from the actively immunized mother little if any hemolytic immune-body. We must therefore direct our attention particularly to the antibody bearing colostrum-milk, and the gastro-intestinal absorption of antibodies by the young.

Observations upon the colostrum, taken at time of parturition, and before the young were permitted to suckle, proved to be very interesting. When compared with the same animal's blood serum, taken at the same time and tested in parallel, it was sometimes found to be considerably higher in immune-body value, in some instances reaching a value two to three times greater than the blood serum. The published data of other workers would indicate that this occurs not infrequently where animals are immunized, some time before birth of young, against various antigens (Stäubli,[32, 80] Wegelius[24]). But particular attention has not been given to its importance. The question arises, Is the high hemolytic

value of the colostrum in these cases due to (1) accumulated specific antibodies formed primarily in the mammary gland, or (2) a storage of antibody removed from the blood stream, or (3) are these antibodies present only in a concentration equivalent to that of the other body fluids but are augmented in action by other substances present in the colostrum, e.g., substances analogous to the "bovine colloid" of Bordet and Gay[72]?

As yet we know little concerning which tissues or organs form the hemolysins. The theory has been advanced, with some experimental support, that the blood-forming organs are the principal factors in the production of antibodies (Hektoen[73]). According to Hektoen,[69, 71] the blood itself, and probably the subcutaneous tissues at site of injection of blood corpuscles, do not fix the antigen and produce hemolysins to any appreciable extent. If this view concerning the formation of antibodies is accepted, little reason exists to suppose that the hemolysins are being formed to any extent in the mammary gland. No experimental proof has been brought forward showing that the cells of secreting glands produce antibodies. So, on the whole, little evidence exists which would support the first possibility.

Probably the second possibility mentioned is the true explanation of what actually occurs. In the absence of any direct experimental work on this particular question, certain facts may be considered, and a hypothesis deducted which will support the view. The quantitative relationship between the protein constituents of normal colostrum, and normal milk, in conjunction with known facts, must be considered. In the absence of full chemical data upon goat's colostrum and milk, the data upon analogous products from the cow must be used. According to König's[75] tables, the composition of goat's and cow's milk is very similar. Hoppe-Seyler-Thierfelder[76] states that colostrum is probably somewhat poorer in casein than milk, but very much richer in albumin and globulin. König's tables show practically the same general results with the exception of the casein, which he gives in a higher percentage for the average colostrum (4 per cent) than in the milk (3 per cent); he states as the average for coagulable protein (albumin and globulin) in colostrum, 13.6 per cent—for milk (albumin)

0.53 per cent. The colostrum contains an especially high content of globulin (Eichelberg[77]), while the milk contains only traces of this protein. At once we are struck by the great extremes of protein content existing between the colostrum and the milk from the cow. But it is also noticeable that cow's colostrum and milk do not show this great quantitative variation between their casein contents. Some observers report that the average casein content is about the same in colostrum and later milk. Casein is considered to be a nucleo-albumin, which is elaborated by the glandular epithelium and according to Mandel[78] probably originates in the gland cells from a breaking down of the nucleo-protein. So the casein content of the colostrum or milk may be used as an index of the secretory activity of the gland. The lacto-albumin which is present in the milk is also probably elaborated in the gland cells, and, with the casein, constitutes the normal milk proteins. The trace of lacto-globulin present in later milk has the properties of the sero-globulin, and probably is simply excreted by the gland cells by removal from the blood stream in an unchanged condition. In support of this supposition the work of Bauer[79] may be quoted. He prepared anti-sera by the injections of serum, colostrum, and milk. By a refined complement fixation test he was able clearly to differentiate blood serum and milk from the same animal. But when the anti-serum from cow colostrum was used, he found on testing that the highest specificity was shown against the same colostrum ($\frac{1}{100,000}$) but also he had a fixation with cow's milk ($\frac{1}{10,000}$) and ox serum ($\frac{1}{10,000}$). These results showed that in colostrum there is present, not only the special milk proteins, but also those peculiar to the same animal's blood serum. His experiments would indicate also that the milk proteins were a specialized product of the milk glands. From both the purely chemical and the biologial reaction standpoint, it would appear that the proteins of colostrum and milk are somewhat different in qualitative character and in origin. The colostrum contains, in addition to those proteins of the normal milk, blood serum proteins, especially globulin, in considerable quantity.

It is a well known fact that during the active immunization of an animal its blood serum shows a decided increase in its protein

content.* Also it is equally well known that antibodies themselves, if not protein in character, are closely associated with the proteins—in particular the serum globulins. Since we have seen that globulins are present in considerable quantity in colostrum, and that the biological test showed that colostrum contained serum protein while milk did not, we can assume that this is a seroglobulin, as only a trace of globulin is found in normal milk. The high concentration of antibodies in the colostrum might be explained by the excretion of serum proteins (globulin, etc.) by the glandular ephithelium, and incidentally the specific antibodies which are probably closely associated. The antibody value, if the hypothesis is true, would parallel the protein concentration in the colostrum.

Stäubli [32, 80] made similar observations upon typhoid agglutinin-colostrum and blood serum. He found that the colostrum from a clinical case, as well as animal experiments, showed much higher agglutinin content than the blood serum at the same time. He thought that perhaps the milk glands took up the antibody from the blood and returned the water, thus causing a concentration; and also that a certain parallelism existed between the coagulable proteins (lacto-albumin, lacto-globulin) and the agglutinins. He considered the gland cells as playing an important *active* rôle in the formation of agglutinins, and that the high value of colostrum was influenced by the destruction of those cells.

The possibility that substances may be present in colostrum, which might augment the action of the hemolysins, offers some points of theoretical interest. But the supposition is hardly tenable as an explanation, since the dilution of the colostrum was so great in many instances that a "colloidal action" would be negligible. Other chemical substances present would also suffer in the same way. These substances would also appear in the normal colostrum drawn from the animal before immunization, but in no case did we ever get a hemolysis from such a colostrum, in the

*Ledingham (*Jour. Hyg.*, 1907, 7, p. 65) reported quantitative protein changes in serum of a goat during an immunization against diphtheria toxin. The total protein in the normal serum before injection of toxin was 5.867 per cent; the maximum reached during the course of immunization was 8.555 per cent, then gradually diminished. Gibson and Banzhaf (*Jour. Exper. Med.*, 1910, 12, p. 411) never found the total proteins to rise above 10.52 per cent during the active immunization of horses (11 animals) against diphtheria or tetanus toxins.

presence of rabbits or guinea-pig complement, even when used in large quantities.

Before leaving this side of the question we wish to say that it is highly desirable that further work should be carried out along these particular lines.

The gastro-intestinal absorption of unchanged antibodies from immune serum has received attention ever since the introduction of serum therapy. Escherich[81] attempted to passively immunize children by giving antidiphtheritic serum *per os*. He reported that infants of the first months showed a slight rise of antitoxin in their blood, but in older children he met with no success. Laboratory animals failed to absorb appreciable amounts of antitoxin, even when fed in relatively large quantities (Carrière[82]). The work of the v. Behring school (Behring[17], Ransom[57], Roemer[83]) confirmed the findings that adult animals under normal conditions do not absorb antitoxic serum (heterologous) in an unchanged condition, but the newly born animals do so readily from the mother's milk (homologous). They attempted to explain this on the basis of the differences between the anatomical structure of the gastro-intestinal mucosa in the newly born and the grown (Disse[84]), but these claims have not been verified. At the present time certain other factors are considered very important in this process. Salge[85] fed antidiphtheritic serum to infants, but states that no antitoxin passed over to the blood. He observed that infants getting antitoxin through the milk of the nurse readily absorbed it. He considered this difference to be due to homologous antibodies in the milk which were easily absorbed, while the heterologous antidiphtheritic serum was not assimilated in an unaltered state. Salge,[86] in order to test this point, fed infants for several weeks with goat milk (heterologous) drawn from immunized animals and showing considerable antitoxic value, but was unable to demonstrate the presence of antibodies in the blood of the infants. The work of Bertarelli[87] and others would tend to support the same view, although this point is not fully settled.

In my experiments it was seen that newly born kids readily absorbed hemolysins when taking the mother's colostrum-milk containing these bodies. Also in one case, in which the homologous

hemolytic serum (highly potent) was fed, it was absorbed in considerable amounts but probably not so readily as the colostrum-milk hemolysins. Uffenheimer[88] in his extensive work upon gastro-intestinal absorption (bacteria, proteins, antibodies) touched upon the absorption of hemolysins. He fed guinea-pigs, newly born or during the first days of life, considerable amounts of hemolytic serum derived from rabbits immunized against guinea-pig blood cells. He concluded that none of the hemolysins was absorbed, since the young animals gave no evidence of intoxication (hemoglobulinuria, decrease in number of blood cells, etc.). Much smaller doses of the same serum when injected into controls caused their death.

The reports of several investigators (Ganghofner u. Langer,[89] Uffenheimer,[88] Roemer u. Much[90]), upon the question of the gastro-intestinal absorption of unchanged proteins, etc., by young animals, call attention to various factors which influence the absorption, such as age, overfeeding of proteins, the nature and source of the proteins, digestive powers, irritation or lesions of the intestinal mucosa, species of animal, individual variation, etc. In considering the possibility of immunizing the young animal through the digestive tract, the above factors must be kept in mind.

The failure to transmit immune bodies to the suckling when the mother is immunized after its birth, in all probability, is chiefly due to two reasons: first, the milk immune bodies do not appear in the milk at any time in very great quantities; while secondly, the time of their appearance is past the critical age point where the young are able to absorb the hemolysin (unchanged) to any extent.

When immunization against sheep corpuscles was carried out in goats during the period of lactation, the antibody content of the milk when compared with that of the serum was never greater than 1:45–50. Similar observations upon goats immunized against diphtheria and tetanus showed that the milk contained relatively greater amounts of antitoxin when compared with the serum, than shown in the above ratio for hemolysins (Ehrlich u. Wassermann[91]).

All observers are agreed in the conclusion that the newly born animal (or infant) absorbs from the digestive tract unaltered antibodies most readily when given immediately after birth. The

most effective means of supplying the antibodies to the young is through the mother's colostrum-milk (homologous); probably next in importance is the homologous serum, and finally the antibody milk or serum from some other species.

It follows from our observations that of all factors the colostrum plays the most important rôle in transmitting antibodies from the actively immunized mother to the young. Even under normal conditions, it is reasonable to suppose that protective substances are concentrated in the colostrum from the mother's body fluids and may be in part absorbed by the infant after the first feedings. Consequently it seems of highest importance that the newly born child should, during the first days of life, receive the colostrum-milk, unless it is contra-indicated for some special reason. Perhaps the natural resistance which many infants show against many infectious diseases may be partially explained on the basis that the child is passively immunized by anti-substances excreted through the immune mother's colostrum and milk, which are absorbed by nurslings during the early days of life. Such a view gains strength from the observations of Moro,[92] who found that the blood serum from breast-fed infants showed greater bactericidal and hemolytic powers than that from infants who were artificially fed upon cow's milk. The practical application, of adding to the mother's immunity by vaccines during pregnancy, would undoubtedly be attended with risk of disturbing the natural course of pregnancy, and should not be entered into unless further investigation demonstrates its safety.

6. *Summary.*—To summarize briefly the principal results of the foregoing experiments, it was found that:

1. Goats actively immunized against sheep blood corpuscles during gestation passively transmitted the specific hemolysin to their young.

2. The colostrum was the chief agent in bringing about the passive immunization of the suckling. Sucklings which got the colostrum and first milk rapidly acquired a relatively high antibody content in their blood, which was well retained.

3. When the immunization was done during the period of gestation the colostrum contained a high content of specific

hemolysin, often much higher than the adult's serum, at time of parturition.

4. The hemolytic antibodies rapidly disappeared from the milk after the mother had been suckled by the young.

5. The blood taken from the newly born before they were permitted the antibody colostrum showed no appreciable amount of hemolysin by the test used. The placenta played a minor rôle in the passage of hemolysins to young before birth, practically negligible in most cases.

6. Mother goats, actively immunized against sheep-blood corpuscles immediately after birth of their young, failed to transmit any demonstrable immunity to their suckling young.

7. The milk, in some cases, contained no demonstrable hemolysins, but in others showed fairly large amounts.

8. Apparently a very high degree of immunity is necessary before appreciable amounts of antibodies are excreted through the milk.

9. Older sucklings apparently did not absorb the antibodies in an unchanged condition.

10. The young animals (kids) did not respond to any extent in production of hemolysins following subcutaneous injections of foreign blood cells (sheep).

In conclusion, I wish to express my indebtedness to Dr. William H. Park, Director of Laboratories, Department of Health, for many helpful suggestions in connection with the above work. I am also indebted to Drs. Paul Bartholow, and Dr. Maria Grund, for valuable assistance.

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THE RÔLE OF INJURY IN THE PRODUCTION OF A CHICKEN SARCOMA BY A FILTERABLE AGENT *

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The tumor produced by the injection of a Berkefeld filtrate of our transmissible chicken-sarcoma is first noticed as a minute nodule at some point in the track of the injecting needle;[1] and only a small proportion of the fowls injected develop the growth. On the other hand, when the causative agent has been introduced in the form of dried and powdered tumor-tissue, suspended in Ringer's solution, the sarcoma appears as a more or less diffuse mass at the site of injection; and it is found in many of the fowls. These facts have led us to suppose that the filterable causative agent requires for its action a cell-derangement or proliferation, such as the needle-prick or the presence of dried tissue induces. Experiment shows that this is indeed the case.

A number of susceptible fowls were injected, in one "breast" with a large quantity of active filtrate, in the other with an equal quantity of filtrate to which was added a little sterile, washed, diatomaceous earth. Podwyssozki[2] has shown that diatomaceous earth by its mechanical action produces in guinea-pigs a reactive proliferation of the connective-tissue elements; and our controls prove this to be true in chickens as well. The chickens injected with the Berkefeld filtrate were examined every week. Many of them developed a growth where both filtrate and diatomaceous earth had been injected, and in a lesser number a tumor also resulted from the filtrate alone. But in the one instance the tumor regularly arose as a diffuse mass, owing, as examination showed, to a simultaneous proliferation from

* An article somewhat introductory to this one appeared in THE JOURNAL, June 1, 1912.

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. Jour. Exper. Med., 1911, xiii, 397.

2. Podwyssozki, W.: Beitr. z. path. Anat., 1910, xlvii, 270.

many foci; whereas in the other it slowly appeared as a discrete, small nodule in the needle-track.

The effect of the filterable agent when injected into the blood-stream was now taken up. It was found that when a large quantity of an active Berkefeld filtrate, free of foreign particles, was injected into the circulation, a tumor seldom resulted (four cases among seventeen). The sarcoma arose more frequently when a little diatomaceous earth had been added to the filtrate, seven out of twenty fowls then developing a growth. Apart from these figures, the site of the tumors arising after the injection of filtrate free of foreign particles demonstrates the importance of cell-derangement. In three cases the growth had its primary seat in a functioning ovary, where injury and proliferation are of daily occurrence. In the fourth the growth was primary in the liver. Because of the general results, we feel that some focal derangement must have been present in this organ.

The factor of injury will not suffice of itself to explain the sarcoma's striking lack of infectivity under ordinary circumstances. During the past three years we have kept at one time or another in relatively close quarters, over 1,200 chickens, many of them with the sarcoma. To some the fresh, sarcomatous tissue has been fed, and many must have been contaminated with the dried tissue, in which, as we have found, the causative agent will remain active for over seven months. Trauma and other types of injury have been frequent among these chickens, yet not one has developed the sarcoma except when directly inoculated. Furthermore, judging from our recent examination of many "spontaneous" tumors the growth is not naturally endemic among fowls.

The nature of the other factors conditioning the tumor's origin has not yet been determined. It is evident that they are both local and general. Even with the agent in its present active form, and with the factor of injury supplied, many of the fowls injected with a large amount of filtrate fail to develop a growth. When the sarcoma follows an intravenous injection it is seldom primarily multiple, despite the numerous injuries everywhere caused by the infusorial earth. To reach the wing vein for injection we make an incision, thus producing an injury in which the tumor-producing agent might well be expected to localize; yet if the injecting needle is flushed with salt solution before being withdrawn, and

the vein is tied off, the occurrence of a growth at this site is practically ruled out. Our experiments to determine the precise stage of tissue injury or proliferation which favors the occurrence of a tumor have not given clear-cut results.

The conditions determining the incidence of the chicken sarcoma have considerable interest in view of its close resemblance to some malignant mammalian tumors, not only as regards growth and general behavior, but also in its obvious lack of infectivity under ordinary circumstances. This last feature is accounted for in the case of the chicken tumor by the factors on which depends the action of the growth's filterable cause. That injury should be a determining factor is noteworthy in view of its importance as a contributory cause of mammalian sarcomas,' including those of human beings.

Reprinted from The Journal of the American Medical Association
June 8, 1912, Vol. LVIII, p. 1751

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No. 17

THE RELATION BETWEEN A CHICKEN SARCOMA'S BEHAVIOR AND THE GROWTH'S FILTERABLE CAUSE *

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In several previous papers from this laboratory the behavior of a chicken sarcoma transmitted by a filterable agent has been described.[1] Emphasis has been laid on the growth's typically neoplastic character, and on the lack of phenomena suggesting the presence of an extrinsic causative agent. The importance of the subject in its possible bearing on the etiology of mammalian growths has led us to a further experimental study, here to be briefly reported.

An obvious point at which to attack the problem of the relationship between the avian growth and its cause is furnished by the process of metastasis formation. The histologic findings indicate more clearly than in the case of most tumors[2] the development of secondaries of the sarcoma from transported cells. But is this the usual method of the growth's dissemination? And, if so, are not at least some of the metastases due to a localization of the causative agent as such?

To test whether the sarcoma cells, as emboli, can proliferate and give rise to tumors has not been difficult. A suspension in Ringer's solution of particles of the fresh neoplastic tissue was injected intravenously into a number of susceptible, normal fowls, and at short intervals these fowls were killed and portions of the lungs examined in serial section. The mode of formation of the lung-tumors was in this way traced. It was found

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. For the literature see an article by the authors, THE JOURNAL A. M. A., June 1, 1912, p. 1682.

2. Rous, Peyton: Jour. Exper. Med., 1911, xiii, 397.

that they uniformly arose by a survival and growth of the injected tumor cells, with penetration of the vessel-wall and extension into the surrounding tissue. The histologic pictures strikingly illustrate the development of a tumor by the multiplication of cells already neoplastic.

The visceral metastases of the sarcoma, occurring in the ordinary course of the disease, appear first and most frequently in the lungs. This rule is subject to exception much more rarely than in the case of human tumors metastasizing by the blood-stream. In only five of 157 fowls with visceral metastases were the lungs free of sarcoma and the further organs affected. In these cases, unfortunately, a patent foramen ovale was not looked for; but in two similar ones occurring since these statistics were compiled, the abnormality was present. Forty-one of the 157 fowls had metastases in the lungs alone; in 111 the lungs and other viscera were affected, often with very numerous secondary growths. In connection with the foregoing experiments, such a distribution is strong evidence for a general origin of the metastases from cell-emboli, which latter, as usual, are sieved out of the circulation by the pulmonary capillaries. There is, however, the alternate possibility that the extrinsic agent, as such, engenders tumors more easily in the lungs than in other organs. That this is not the case is shown by the results of direct intravenous injection of a Berkefeld filtrate containing the causative agent. In only two of eleven fowls developing a growth after such treatment was the sarcoma primary in the lungs, despite the fact that the agent was carried in bulk directly to these organs. In seven of the cases diatomaceous earth had been added to the filtrate and had lodged for the most part in the pulmonary capillaries, there producing injury such as has been found to favor the agent's action.[3]

Although it is thus certain that the chicken sarcoma's metastases are in general referable to a development of cell emboli, there remains the question whether secondary tumors may not be caused occasionally by the filterable agent as such. That the agent can enter the circulation is demonstrated by the appearance in some instances of a sarcoma after the injection into susceptible hosts of large quantities of centrifugated, "paraffin" plasma from

3. The Journal A. M. A., June 8, 1912, p. 1751.

fowls moribund with the metastasizing growth. The general importance of injury in determining the agent's action led us to attempt to obtain by its means secondary tumors under conditions which would show them to be independent in origin of transported cells.

Tissue derangements in tumor-bearing fowls were produced by the injection of scarlet red, or diatomaceous earth, or by incisions allowed to heal by secondary intention. The numerous experiments have given results almost entirely negative. Usually the sarcoma failed to localize at the seat of injury. A single case suggests the direct action of the extrinsic agent. In a chicken with a very large primary sarcoma, but no discoverable metastases in the viscera, a small secondary growth was found post mortem at the site of injection of diatomaceous earth. The heart was not examined for a foramen ovale. Two other instances of localization at the point of injury are less significant because of the presence of many metastases in the lungs and elsewhere. A third and similar case has come to attention during the routine autopsies on several hundred tumor-bearing fowls. The chicken had a large primary growth at the site of inoculation in the pectoral muscles, many metastases in the lungs but none in any other organ except the oviduct, in which were two small nodules. The oviduct contained an inspissated egg, and its walls showed reactive thickening and increased vascularization. Whether in any or all of these cases there was an action of the extrinsic agent, as such, cannot be said. When metastases develop at sites of injury in human beings the morphology of the growths often shows their origin from transported cells; but this feature is of no assistance with a spindle-celled sarcoma, such as ours, which might be primary anywhere.

The subsidiary part taken by the causative agent in the sarcoma's dissemination, if indeed it takes any direct part at all, is in keeping with what is already known of the tumor's growth and general behavior.[1] Yet in the end the sarcoma must be closely dependent on the activity of its filterable cause, for when this latter has been attenuated by a long stay in glycerin the tumors resulting from its inoculation develop slowly and frequently retrogress.

The conditions which determine the curious relationship between the disease and its cause are of much inter-

est. How does it happen that the sarcoma, though ultimately dependent on an extrinsic agent, is dominated in its behavior by the cells composing it? Some simple reasons suggest themselves. In the first place, the agent's action to produce a neoplastic change takes place with extreme slowness as compared with the proliferation of the cells, once they have become neoplastic. Consequently the growth of the neoplasm appears to take place entirely by multiplication of the cells of an initial focus. Growth doubtless does take place wholly in this way in many cases because of a second peculiarity of the agent, namely, its dependence on a special set of conditions in order that it may produce a neoplastic change.[3] The necessity for such conditions goes far to explain the agent's failure to take an active part in the tumor's dissemination in the body, granting indeed that the agent is present in the circulation before the fowl is moribund. The possibility of immune processes effective against the agent when separate from the cells must be kept in mind. The immune processes thus far recognized have been directed against the cells themselves, as in the case of the mammalian tumors.

The relationship existing between the chicken sarcoma and its cause, as shown by previous papers and by the findings here presented, seems to us to furnish some basis for the conception of an extrinsic cause for other sarcomata.

Sixty-Sixth Street and Avenue A.

Reprinted from The Journal of the American Medical Association
June 15, 1912, Vol. LVIII, pp. 1840 and 1841

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THE NATURE OF THE FILTERABLE AGENT CAUSING A SARCOMA OF THE FOWL*

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Although the filterable viruses have but recently come to attention, it is known that they are of very diverse character and that, except as a matter of expediency, they can scarcely be discussed together. At present each constitutes a separate problem. This is especially true of the filterable agent which causes a sarcoma of the fowl.[1] The disease is so different from the ordinary infectious processes that nothing can be preassumed with regard to the character of the etiologic agent. On the contrary, in considering it one must take into account all those numerous and often bizarre possibilities which are suggested by the literature on tumor-causation.

The first question concerning the agent is whether it is living. A very little of it will give rise to a growth from which numerous others may be started, each yielding the agent in abundance. But this does not necessarily settle the point. The agent might very well be a chemical substance somewhat similar to those used experimentally to produce atypical cell-proliferation. The fact that the proliferation induced by a chemical compound ceases as the latter is expanded, whereas our sarcoma's growth is continuous, constitutes no objection. For the sarcoma cells may themselves be the source of the inciting chemical, and thus serve to perpetuate the disease. Such an assumption throws little light on the origin of the "spontaneous" tumor which by transplantation has yielded our material.

The most direct means of proving that the agent is alive is to grow and transfer it in culture. Recently we

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. Rous, Peyton: The Journal A. M. A., Jan. 21, 1911, p. 198.

have done much work in this direction without success. The results of differential filtration may have some value as indicating whether the agent is a formed body and its probable size. Here our recent results confirm those first reported. In a dilute tumor extract the agent will pass through Berkefeld filters which hold back at the same filtration *Bacillus fluorescens liquefaciens,* an organism measuring 0.5 micron by 1.0 to 1.5 microns. On the other hand, Chamberland bougies (F) prove impermeable despite conditions which seem very favorable to passage. Could the findings be accepted at their face value, they would go to show that the agent is organized and perhaps even visible. But many factors serve to invalidate negative results with filters of fine texture. As is well known, these may hold back the complex proteins as well as living organisms. Nevertheless, in view of the findings, we have made repeated attempts to demonstrate the agent with special stains or the dark-field microscope, but to no purpose.

The conditions under which the agent can survive have much interest. As elsewhere stated, it may retain its activity in dried tissue for seven months, and for at least one month in tissue placed in 50 per cent. glycerin. In both instances it undergoes a gradual attenuation, as evidenced by the behavior of the tumors it engenders. These appear in relatively few hosts and after a long interval; they grow slowly and retrogress frequently. The process of repeated rapid freezing and thawing, which reduces the tumor tissue to a pulp, does not markedly lessen the activity of the associated agent. The latter's resistance to heat, on the other hand, is little greater than that of the tumor-tissue itself, as has been shown by parallel inoculations of bits of the same heated material into susceptible fowls and the plasmatic medium of Burrows. Tumor-tissue submitted to a heat of 50 C. (122 F.) for fifteen minutes fails absolutely of growth in the plasmatic medium, in which its proliferation is ordinarily very active; but when inoculated into susceptible fowls it often gives rise to tumors and may do so even when it has been heated at 53 C. (127.4 F.). Whether growth in the plasmatic medium is a real index of the tumor tissue's viability is uncertain. But that the filterable agent will withstand a temperature of 50 C. for fifteen minutes is shown by the production of tumors with tissue which has been dried, ground and suspended in Ringer's solution previous to heating.

Material which has been heated to 55 C. (131 F.) for fifteen minutes never gives rise to the sarcoma.

In sarcomatous tissue autolyzing at the temperature of the chicken's body (41 C., 105.8 F.) the agent remains active for less than forty-eight hours. Toluol and chloroform in the proportions employed to prevent bacterial growth during autolysis destroy it in less than two hours. So, too, will 50 per cent. alcohol or 2 per cent. phenol (carbolic acid). Unlike the virus of poliomyelitis, the agent fails to withstand phenol 0.5 per cent. Like the animal organisms in distinction from most of the vegetable ones (von Prowacek) it is rapidly destroyed by bile, and by saponin in high dilutions. At 41 C. it is within two hours deprived of all activity by 50 per cent. rabbit bile or chicken bile, or by saponin in strengths greater than 1 to 800.

To obtain the foregoing data the only test employed, or at this time possible to employ in demonstrating the agent's activity, has been the inoculation of susceptible fowls. No single attribute among those determined suffices to show the nature of the agent; yet, taken together, its characters are those which we associate with microorganisms.

Reprinted from The Journal of the American Medical Association
June 22, 1912, Vol. LVIII, p. 1938

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NO. 19

A CLINICAL STUDY OF ACUTE POLIOMYELITIS

BY

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REPRINTED FROM
MONOGRAPH OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH. NUMBER 4.
JUNE 1, 1912.

A CLINICAL STUDY OF ACUTE POLIOMYELITIS.

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(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

Plates 1–13.

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The following study is a report of the work on acute poliomyelitis carried on at the Hospital of The Rockefeller Institute for Medical Research during the summer of 1911. The material consisted of one hundred and sixty-one cases which occurred during the present season and twenty-two cases from previous years. Seventy-one of these cases were admitted to the hospital and were

under observation for a period averaging from three to four weeks. The other cases were seen in the dispensary.

The study was chiefly of a biological nature, and, on account of lack of time, observations of the disturbances of the reaction of the muscles to electrical stimulation were used for diagnosis and prognosis to a limited extent only.

We have attempted to correlate our experience with that of other observers, but no effort has been made to give a complete review of the literature. For a complete bibliography we refer the reader to the articles and monographs on which we have drawn freely.[1]

INTRODUCTION.

The recognition of poliomyelitis as an acute infectious disease was delayed largely owing to the strange relationship between the obscure, inconsequential features of the early stage and the flagrant character of the resultant paralyses. In most text-books, descriptions of the disease as an acute infection dismiss the fever and initial malaise in a few lines, while the diagnosis is declared easy because of the sudden and complete paralysis. The following words from Allbutt's System of Medicine (1905) are significant: ". . . and after two days, when the pain had passed away, the case was found to be one of infantile paralysis. The fever and general constitutional disturbances at the onset had obscured the diagnosis." Furthermore, as a result of the earlier pathological studies of infantile paralysis,

[1] Römer, P. H., Die epidemische Kinderlähmung, Berlin, 1911.

Wickman, Ivar, Beiträge zur Kenntnis der Heine-Medinschen Krankheit, Berlin, 1907; Die akute Poliomyelitis, bzw., Heine-Medinsche Krankheit, Berlin, 1911.

Zappert, von Wiesner, and Leiner, Studien über die Heine-Medinsche Krankheit, Leipzig and Vienna, 1911.

Müller, E., Die spinale Kinderlähmung, Berlin, 1910.

Harbitz and Scheel, Pathologisch-anatomische Untersuchungen über akute Poliomyelitis und verwandte Krankheiten, Christiania, 1907.

Flexner, The Contribution of Experimental to Human Poliomyelitis, *Jour. Am. Med. Assn.*, 1910, lv, 1105.

Massachusetts State Board of Health, Infantile Paralysis in Massachusetts in 1909, Boston, 1910.

Epidemic Poliomyelitis. Report on the New York Epidemic of 1907 of the Collective Investigation Committee, New York, 1910.

made chiefly upon cases that died later from other causes, the term atrophic was added to the obscuring nomenclature of the disease. The changes noted in the spinal cord were scar formations in the anterior horns, and no lesions were found elsewhere in the body. Consequently, attention was focussed on certain chronic changes in a limited portion of the spinal cord. From the practitioner's standpoint, the natural course of the malady is such that his efforts have been palliative rather than curative. Usually he is called to face, helplessly, a child recovering from a febrile disturbance, but stricken with an amazing paralysis. The crippled state of the patient becomes the all-engrossing feature of the case, and the physician's chief effort is to devise methods of support and artificial activity. The management of this disease, therefore, has become a problem for the orthopedic surgeon; the internist is too late.

Fortunately, however, the work of the past few years on the experimentally produced disease, and, above all, the fine accounts of the human affliction by Wickman, Müller, and others have been most illuminating. It has become clear that the first outward expression of the disease is the fever, and in order that the subsequent paralyses may surely be prevented, the infecting agent must be destroyed before it has called forth a damaging reaction in the body. In the event of possible means of treatment in the future, therefore, early diagnosis is imperative, and in view of the obscure and often insignificant mode of onset, the problem becomes one of great difficulty, which calls for the best qualities and equipment of the internist. In those very symptoms of onset, the fever and general constitutional disturbances, which have been said to obscure the diagnosis, lie the warnings which should arouse the physician at once to such special procedures as are known to be helpful in making the diagnosis.

Besides the importance to the affected individual, the recognition of acute poliomyelitis in its febrile stage has a serious bearing on the community at large. It has been definitely shown that there are many obscure cases that never develop paralysis, whose mild symptoms indicate no specific infection, but which may carry and spread contagion. Just what proportion of all cases these abortive cases form is still a doubtful point. In the large epidemics, where

physicians are expectant, the reported number of such cases is relatively larger than among a series of sporadic instances of the disease.

At all events, it seems evident that abortive cases play a most important part in the total incidence of the disease; and in connection with their great frequency, it must be borne in mind that the virus is probably present in these cases in as active a form as in the patients with paralysis. In view of these considerations, it is obviously a matter of great importance to determine the relative frequency of the abortive forms of the disease, and to recognize them, for it is these cases which, not being recognized, escape quarantine and spread the virus. In general, the cases that develop no paralysis are clinically similar as regards history, prodromata, and onset, to those that go on to disability. Early diagnosis, therefore, serves a double purpose. The individual may, with an effective therapy, be spared a paralytic visitation, and the community may be protected from the menace of an unrestricted virus carrier.

The purpose, then, of the following pages is to emphasize the fact that poliomyelitis is an acute disease, in which the often insignificant febrile stage is of high epidemiologic and therapeutic importance.

HISTORICAL.

During the past seventy years infantile paralysis, or acute poliomyelitis, has been gradually separated from a mass of heterogeneous palsies and established as a pathological and clinical entity. Although between forty and fifty epidemics have been described, it is a striking fact that the first three important milestones on the path of our advancing knowledge of the malady are separated by long intervals of time.

In 1840, Heine,[2] an orthopedic surgeon of Cannstadt in Germany, published a monograph on paralytic conditions of the lower extremities and their management. The chief stress of the work is laid upon the surgical aspect of the disease, but there is a short, clear cut description of the onset and acute stage. For etiology,

[2] Heine, Jacob, Beobachtungen über Lähmungszustande der untern Extremitäten und deren Behandlungen, Stuttgart, 1840; Spinale Kinderlähmung, Stuttgart, 1860.

a sudden serous exudation in the spinal cord is suggested, and a rough description of spinal cord changes indicates the author's idea of the site of the lesion. The work of Heine is a fine example of keen clinical observation and leaves no doubt of the correctness of his view that the disease is an entity. Except for an epidemic described by Colmer,[3] there is little more of clinical importance in the literature of poliomyelitis for the next forty years. Charcot,[4] in 1870, studied the pathology of poliomyelitis and declared a primary degeneration of the anterior horn cells to be the morbid process. This view has been the subject of much discussion. In 1884, Strümpell[5] pointed out the resemblance between certain cases of acute encephalitis with spastic paralysis in children and cases of poliomyelitis. He concluded that these two types of disease of the central nervous system had a common etiological factor, and suggested for the first time the hypothesis that an external infectious agent was responsible for them. His recognition of the true cerebral form of the disease was a most important contribution to the knowledge of poliomyelitis. During the numerous epidemics reported in the following years, many instances of apparently direct contagion were observed, which added support to Strümpell's suggestion. There was still lacking, however, a description of the symptomatology of the disease in its complete course.

In 1890, Medin,[6] basing his studies upon the extensive Swedish epidemics, published the first good clinical account of acute poliomyelitis. Medin's work is perhaps as much of a classic as Heine's, and his name has been coupled with the latter's in association with the disease. He likewise described the spastic types of poliomyelitis which had been recognized by Strümpell as occurring in association with the usual forms. Following this, almost each year saw reports of smaller epidemics in France, Italy, Germany, and the United States; but no important additions to the existing knowledge of the disease were made, until Wickman,[7] in 1905, studying a vast amount

[3] Colmer, *Am. Jour. Med. Sc.*, 1843, v, 248.

[4] Charcot, quoted by Oppenheim, Lehrbuch der Nervenkrankheiten, Berlin, 1908.

[5] Strümpell, *Jahrb. f. Kinderheilk.*, 1884, xxii, 173.

[6] Medin, *Verhandl. d. X. internat. med. Cong.*, 1890, ii, 6 Abt., 37.

[7] Wickman, Beiträge zur Kenntnis der Heine-Medinschen Krankheit, *loc. cit.*

of material in Sweden, developed the epidemiology of the disease and its pathology, and described for the first time the abortive types. The importance of this particular contribution will become more evident in the discussion of the epidemiology. Wickman's exhaustive studies demonstrated conclusively the contagious nature of the disease. There remained, therefore, the problem of finding the infecting agent.

Experimental production of the disease in monkeys was first accomplished in 1909 by Landsteiner and Popper,[8] and a few months later by Flexner and Lewis[9] and by Strauss[10] in this country. Three observers in different cities independently succeeded in transferring the disease from one monkey to another. This was done in November, 1909, by Flexner and Lewis in New York, Leiner and von Wiesner[11] in Vienna, and Landsteiner and Levaditi[12] in Paris. The reports from the various sets of workers appeared within a period of two weeks.

As a result of the extensive work which has been done on the experimental disease, many important facts about the etiology have been acquired. A bacterial cause has been definitely ruled out and there is now undoubted evidence that the infecting agent belongs to the group of so-called filterable viruses.[13] The virus of poliomyelitis is highly resistant to many destructive measures; thus, it withstands glycerination for long periods and is not injured by 0.5 per cent. carbolic acid. Furthermore, freezing at —2° to —4° C. for forty days does not affect it materially. To heat, it is less resistant and it can be destroyed by a temperature of 45° to 50° C. for half an hour. The virus is readily destroyed by hydrogen peroxide in 2 per cent. solution, by menthol, and by corrosive sublimate. In monkeys, one attack of the experimental disease prevents a second successful inoculation. Fairly satisfactory results in the production of active immunity, induced by repeated injections of small amounts of attenuated virus, have been obtained, but these

[8] Landsteiner and Popper, *Ztschr. f. Immunitätsforsch., Orig.*, 1909, ii, 377.

[9] Flexner and Lewis, *Jour. Am. Med. Assn.*, 1909, liii, 1639.

[10] Strauss and Huntoon, *N. Y. Med. Jour.*, 1910, xci, 64.

[11] Leiner and von Wiesner, *Wien. klin. Wchnschr.*, 1909, xxii, 1698.

[12] Landsteiner and Levaditi, *Compt. rend. Soc. de biol.*, 1909, lxvii, 592.

[13] Landsteiner and Levaditi, *loc. cit.;* Flexner and Lewis, *Jour. Am. Med. Assn.*, 1909, liii, 2095.

have not warranted the application of this procedure in man. The serum of recovered monkeys, and also the serum of human patients that have recovered from this disease, have been shown to contain a substance that is capable of neutralizing the virus *in vitro*. If 0.1 of a cubic centimeter of the active virus[14] and one cubic centimeter of the serum of a case of poliomyelitis be mixed in a test tube, incubated for two hours at 37° C., refrigerated over night and then injected intracerebrally into a monkey, the animal does not have any symptoms of the disease. This neutralizing substance has also been demonstrated in the blood of abortive human cases.

EPIDEMIOLOGY.

The study of the epidemiology or mode of transmission of epidemic poliomyelitis has been much promoted by the observations of Wickman in 1905, who first drew special attention to its contagious character. All subsequent studies have been influenced by the fundamental observations of Wickman, and have tended to support his deductions.

An entirely new light was thrown on the obscure question of dissemination by Wickman's recognition of the so-called abortive and meningitic forms or types of the affection. Until then, attention had been riveted on the paralysis, and the possibility of cases of epidemic poliomyelitis occurring in which paralysis is entirely absent seems not to have been entertained. It is at once clear that the existence of such cases would throw an entirely new light upon the mode of transmission of the infection.

That epidemic poliomyelitis must be an infective process follows from its epidemic character. We are unacquainted with any disease that flourishes in epidemics that is due to other causes than parasitic. That the infective cause finds a considerable degree of resistance naturally present in any community is proven by the strong tendency of the frankly paralyzed types of the disease to appear as isolated cases in a given family or household. But this is merely a tendency, since the occurrence of dual and even multiple infection in one household is by no means uncommon. Indeed, now that the abortive and meningitic forms of the affection, without paralysis,

[14] The active virus, so-called, is merely a filtrate of a 5 per cent. salt solution suspension of the spinal cord of an infected and paralyzed monkey.

are acknowledged, the instances of multiple cases are not so infrequent as they were believed to be.

To demonstrate that more than one case may frequently occur in the same family, the following three instances are significant. Two of these involve bottle-fed infants, the other deals with older children.

Family I.—Belle and Clara T., twins, eight months old.

Belle T. Feverish, August 18; next day temperature 103° F., throat red and inflamed, slight patch on the right tonsil; patient nervous; breathing rapid; pneumonia suspected. August 20, child less nervous and began to seem dull. August 21, twitching of face and athetoid movements of foot; later in the day weakness in left arm and leg. August 23, involvement of respiratory muscles; death.

Clara T. August 21, child irritable, vomited several times, and coughed. August 22, temperature 102° F., vomited several times; examination of right leg caused unusually loud screaming; slight weakness in right thigh. August 23, paralysis definite.

Here is a situation where twin infants were infected so nearly synchronously that it is impossible to say whether one case contracted the disease from the other, or whether a common source provided the virus for both. The fact that both infants were bottle-fed makes infection by interchange of nipples conceivable. The next example of family contagion also presents a somewhat similar situation.

Family II.—Margaret W., age fourteen months, began on October 4 to have typical symptoms of onset and in three days developed incomplete paralysis of both lower extremities. Four days later her infant sister became feverish and looked sick. Two days later the latter was brought to the hospital with beginning paralyses which advanced rapidly and terminated fatally by involvement of the respiratory muscles. The mother said that this child had frequently been put in the same bed with the older one, and that she had seen the children alternately suck the nipple of the common feeding bottle.

Family III.—Hannah T., age four years. On October 1, she was well in the morning but vomited in the afternoon and was feverish. The next day she was paralyzed. There are four other children in the family, one older and three younger than Hannah. One week before her illness the older brother had had tonsillitis, and a day or two after his attack began, the three younger children had what the mother called a "drowsy sickness," in one case with fever. A small brother, one of the three, was brought to the hospital with typical symptoms of the acute stage of the disease and significant changes in his spinal fluid. There were no definite paralyses, but a distinct shakiness in walking was noticed.

These family infections speak for themselves in the matter of direct contagion. The last instance, although lacking definite proof,

is highly suggestive of the importance of abortive cases. One further example, however, where the evidence of a neutralization test made probable the correctness of the diagnosis of acute poliomyelitis in a case that had no muscular weakness, is of interest at this time.

Helen K., age three years and ten months. On August 16, she had headache and was feverish; on the 18th, paralysis of the legs began and ascended rapidly to the arms and terminated fatally by respiratory involvement on August 21. Her constant playmate, Helen B., age six years, had headache on September 1, that is about ten days after Helen K. was ill, and the following day was brought to the hospital. Examination of the spinal fluid showed changes similar to those seen in poliomyelitis. She never developed paralysis and after fifteen days went home apparently well. Her blood serum protected a monkey against 0.1 c.c. of the virus.

This child had no definite symptoms beyond some stiffness of the neck, headache, apathy, and anorexia. Except for her close association with the fatal case, her mother would have paid no attention to what might have passed as a slight summer ailment, and a diagnosis would never have been made. The child would have gone to school in a few days and perhaps have been the source of infection for other individuals.

Human beings are very unequally subject to the effects of different contagious affections. It is only smallpox and measles that can vegetate in any soil; all other direct infections are conditioned by degrees of susceptibility of the host that determine the extent of their prevalence. Scarlet fever, typhoid fever, and epidemic meningitis can claim far fewer victims. Indeed, the degree of susceptibility, as determined by age and other circumstances, is strikingly similar in epidemic meningitis and epidemic poliomyelitis.

Epidemic poliomyelitis has been clearly observed to follow the lines of human contact and travel. Wickman has placed the primary center of infection and transmission in the village school, and the secondary centers he has traced to contacts that arise out of circumstances of ordinary human intercourse. Through these, the infection is spread in a village and from one village to an adjacent one. Moreover, he has emphasized that the carrying of the infection is twofold, by the affected and the non-affected, or actively and passively, as one would now say. The most important active

carrier of the epidemic is the victim of an abortive attack of the disease, since he may not be confined during any period and is as capable, probably, of distributing the infection as a case attended by paralysis. This is equally true of some other contagious diseases, notably diphtheria and scarlet fever. For in these diseases which are not highly contagious, as is measles, the healthy carrier has never been given much consideration; indeed it has been largely overlooked. This omission has been due to the high natural resistance possessed by the community.

Attention is then to be fastened upon the human agency of transmission. This is all important because, if it is the true and most frequent agency, then preventive measures, effecting both limitation and final suppression, will be taken accordingly. The admission of this source of contagion will dispose of the vague notions regarding the existence of sources of infection among the lower animals.

The following extract from a paper of Flexner's[15] on the control of epidemic poliomyelitis will serve to show the present state of knowledge and conjecture on this aspect of the problem:

"Paralytic diseases among domestic animals are known and not highly infrequent. They have been noted among dogs, horses, and fowl, but thus far it has not been found possible to correlate the paralytic diseases of the lower animals and those of man. Perhaps the most frequently observed coincidental paralytic diseases have been between hens and human beings. Undoubtedly since the wide prevalence of epidemic poliomyelitis, the existence of a paralytic disease among barnyard fowl has been more commonly noted. Possibly the condition has not actually become more frequent, but owing to the circumstance mentioned it has been oftener observed. It would appear that the paralysis among fowl is caused not by lesions of the central nervous system, but by lesions of the peripheral nerves and that it is due to a peripheral neuritis. It has not been found possible to transmit by direct inoculation the paralytic disease from chicken to chicken, or from chicken to monkey, or from paralytic monkey to chicken (Flexner and Lewis). However, it has been found possible to develop the paralysis in the laboratory by keeping the chickens in confinement for some time, and by supplying them

[15] Flexner, *Am. Jour. Dis. Child.*, 1911, ii, 96.

an unusual and doubtless improper form of food (Flexner and Clark). It has proved as little possible to transfer the paralytic affection of dogs from one individual to another by direct inoculation or from dog to monkey or from paralyzed monkey to dog (Flexner and Lewis). These failures do not, of course, exclude the possibility that a reservoir for the virus may exist among domesticated animals that do not even respond to its presence by developing paralysis or other conditions which could be recognized as resembling poliomyelitis in man. The manner of the action of the virus of poliomyelitis in rabbits provides an illustration which shows how necessary it is to avoid general deductions in this field. At first it was strenuously denied that rabbits could be infected at all with the virus of poliomyelitis, and the examples of supposed successful inoculation reported were entirely disbelieved (Krause and Meinicke, Lentz and Huntemüller); but it must now be accepted that young rabbits occasionally, but by no means generally, are subject to inoculation with the virus of poliomyelitis, at least after it has passed through a long series of monkeys (Marks). Apparently a small percentage only of the inoculated rabbits develop any obvious symptoms, and these die, as a rule, during convulsive seizures which come on suddenly. A given virus has up to the present been sent through a series of six rabbits, after which it has failed to be further propagated. From the sixth series it has been reimplanted on the monkey, in which animal typical paralysis has been produced (Marks). It remains to add that the rabbits which succumb to the inoculation do not show any characteristic lesion of the central nervous system or other organs, as far as has been determined. The monkey, on the other hand, invariably shows the typical lesions of the central nervous system.

"Insect contamination with the virus would serve, were it proved not only to be an experimental possibility but to occur in nature, to clear away any present apparent discrepancies in the epidemiology of the disease. In this connection it should be stated that not only does epidemic poliomyelitis spread over a wide territory, but its spread is not promiscuous, but along the routes of human travel. Therefore insects that seek human habitations and routes of travel,

that possess the power to migrate over a considerable territory, that affect all classes of society, that abound during the period of greatest prevalence of the disease, and that do not wholly disappear at any season, should be the first to come under suspicion. Many, if not all, of these conditions are fulfilled by the common house-fly."

The effort to make out a case for insect transmission has, however, failed up to the present. The part that the house-fly may play in the transfer of the virus is strictly passive. That these insects can carry the virus in a living and actively infectious state for forty-eight hours or longer has been proven by the laboratory experiments of Flexner and Clark. They can, therefore, conceivably accomplish a similar transportation of the virus, as a contamination, in natural infection. Their exclusion from poliomyelitis patients should be zealously carried out.

But the main problem relates to the human carrier, active or passive. Where does he harbor the virus, and how is it taken up by the victims? The evidence is growing constantly stronger that the upper respiratory tract, as early pointed out by Flexner and Lewis, is the site both of ingress and of egress of the virus.

That the lymphatics of the nasal mucosa are in almost direct connection with the subarachnoid space has been clearly demonstrated. Flexner[16] has shown in the case of *Diplococcus intracellularis* that when monkeys are infected by intraspinal injection of cultures, the diplococcus can be found subsequently both in leukocytes and free in the nasopharynx. This fact and the demonstration of the virus of poliomyelitis in the nasal mucosa of infected monkeys point clearly to the nasopharynx as a path of discharge for agents infecting the cerebrospinal space. Successful inoculations of the nasopharyngeal mucosa of monkeys with poliomyelitis virus have shown, furthermore, that the same path may serve to admit the infecting agent.

In addition to the nasopharyngeal mucosa as a source for the distribution of the virus to the outer world, Landsteiner, Levaditi, and Pastia,[17] and Flexner and Clark[18] have shown that tonsils from fatal human cases also contain the virus of poliomyelitis.

[16] Flexner, *Jour. Am. Med. Assn.*, 1910, lv, 1105.

[17] Landsteiner, Levaditi, and Pastia, *Sem. méd.*, 1911, xxi, 296.

[18] Flexner and Clark, *Jour. Am. Med. Assn.*, 1911, lvii, 1685.

The virus of poliomyelitis is unknown apart from infected human beings and monkeys, and yet it possesses a high degree of resistance. It withstands low degrees of cold and ordinary degrees of heat for long periods of time. When enclosed in albuminous matter, it withstands drying for several weeks. It can therefore survive both in moist and dry conditions and thus is capable of being carried directly to the upper respiratory mucous membrane as spray produced by coughing and sneezing and even loud speaking of the infected, and as dust. A more detailed discussion of the conditions that affect the virus was given in the historical section.

From the evidence at present available, therefore, it is evident that epidemic poliomyelitis is a human-borne, contagious affection, the portal of entry for the virus of which is the upper respiratory tract, in particular, the nasopharyngeal mucous membrane. Other agencies than man play a part in distribution that is distinctly subordinate and inferior. The infection can be carried and implanted both by active and passive carriers; being resistant and having access to external nature with mucous secretions, it can become attached to dead objects, bedding, clothing, etc., and to domestic pets and domestic insects (fly); and it can be ground into dust and conceivably be disseminated by wind. Recent experiments of Neustaedter and Thro[19] lend support to this latter hypothesis. These being the probable modes of transmission, preventive measures should be taken accordingly. They are such as are already in use in contagious diseases that have a similar mode of infection; namely, diphtheria and scarlet fever. A further consideration of methods of prevention is given in the section on treatment. Osgood and Lucas[20] have shown that the virus is present in the nasal mucosa of recovered monkeys five months after the acute attack of the disease. A similarly long period of survival of the virus in the mucous membrane of affected human beings has, however, not been demonstrated.

Epidemics rise and fall, and rarely disappear suddenly. The series of cases that form the basis of the present study constitute the instances still arising that owe their initial impulse to the epidemic wave that appeared in New York in 1909, and has since been slowly disappearing.

[19] Neustaedter and Thro, *N. Y. Med. Jour.*, 1911, xciv, 813.

[20] Osgood and Lucas, *Jour. Am. Med. Assn.*, 1911, lvii, 495.

NEUTRALIZATION TEST.

As has been emphasized in the discussion of the epidemiology of poliomyelitis, abortive attacks are of common occurrence when the disease is epidemic. These instances of poliomyelitis are difficult to recognize clinically and a specific biological test would be of great assistance in the diagnosis of such atypical infections. Up to the present time no characteristic reaction has been demonstrated during the acute stage of the disease. Levaditi and Landsteiner, Römer and Joseph, and Flexner and Lewis have shown that the serum of recovered monkeys is able to protect normal animals against fatal doses of the virus. Netter and Levaditi, Flexner and Lewis, and Anderson and Frost have shown that the serum of recovered human cases possesses the same protective property. The last named observers demonstrated this quality in the sera of 66.7 per cent. of a series of abortive instances of poliomyelitis. They also proved that normal human serum has some neutralizing action, but are of the opinion that this action has quantitative limits which clearly differentiate it from the action of the serum of persons who have had poliomyelitis. While such protective bodies do not appear in the serum until about two weeks after the onset of the disease, the fact that the virus is known to survive in the throats of experimental animals for a much longer period of time may make it advisable in extensive epidemics to employ the test in order that the community may be protected from suspected individuals who may act as carriers.

Up to the time of the publication of the paper by Anderson and Frost, the reliability of the test was not questioned. These observers show that normal human serum possesses limited power to neutralize the virus of poliomyelitis. During the past year at the Hospital of The Rockefeller Institute, the serum of a number of individuals was tested for its ability to protect monkeys against infection with the virus of poliomyelitis. The material consisted of sera from normal persons, from those exposed to infection in the hospital ward, from individuals suspected of having an abortive attack of the disease, and from a certain number of typical cases. The test was performed in the following way: the serum was mixed with a fatal dose of a known active virus, incubated from one

to two hours at 37° C. and then allowed to stand for twenty-four hours on ice. The injections were made intracerebrally into monkeys (*Macacus rhesus*). The protocols of the experiments follow.

NORMAL SERA.

Experiment I.—The serum was obtained from a normal adult, one of the physicians in constant attendance on cases of poliomyelitis in the hospital ward. During this time he had presented no signs of illness. One c.c. of the fresh serum was mixed with 0.1 c.c. of virus and was incubated for two hours at 37° C. After standing over night on ice, the mixture was injected intracerebrally into a monkey. After seventeen days the animal began to show signs of weakness and was found dead on the following day. At autopsy the characteristic lesions of poliomyelitis were present in the spinal cord.

Experiment II.—In this instance the serum was obtained from a normal adult, a nurse in charge of cases of poliomyelitis in the hospital. She had no paralyses and gave no history of any illness resembling poliomyelitis, nor was she ill at any time during her service in the poliomyelitis ward. One c.c. of this serum was mixed with 0.1 c.c. of virus and neutralization tested in the manner described. The monkey inoculated became ill on the eighth day after injection. It was somewhat depressed and its movements were slow for several days. On the nineteenth day it developed diarrhea and eight days later it was found dead. At no time did the animal show definite paralyses. At autopsy the microscopic lesions were not characteristic.

Experiment III.—The serum for this experiment was obtained from a girl of eight years of age, a patient in the hospital suffering from chronic endocarditis. She had no paralyses and gave no history of any illness resembling poliomyelitis. Two tests were made with this serum. The first monkey was inoculated with a mixture of 1 c.c. of fresh serum and 0.1 c.c. of virus, prepared in the usual way. The animal survived and at no time developed any symptoms. There was no hyperexcitability and no paralysis. A second test was made with this serum, in which 1 c.c. of serum was mixed with 0.3 c.c. of virus. This animal likewise remained healthy and survived.

Experiment IV.—The serum for this experiment was obtained from one of the doctors in constant attendance on the ward. He gave no previous history of poliomyelitis, but some six weeks previously had a slight illness of about a week's duration, the chief symptoms of which were diarrhea, dizziness, and pain in the neck. There was no muscular weakness. One c.c. of this serum was mixed with 0.1 c.c. of virus and the neutralizing power tested in the usual manner. Seven days after injection, the animal's nutrition was poor; there were no paralyses and no hyperexcitability. On the fortieth day the monkey was still living, when it was again inoculated with 1 c.c. of serum mixed with 0.3 c.c. of virus. The animal developed diarrhea, became emaciated, and died on the eighteenth day after the second injection, without having developed any paralyses.

Experiment V.—The serum of a normal adult, a nurse in attendance on cases of poliomyelitis, was used for this experiment. Some weeks previously she had

a slight illness, lasting three days, the symptoms of which were dizziness and gastro-intestinal disturbance. There was no muscular weakness and no pain associated with the attack. One c.c. of this serum was mixed with 0.1 c.c. of virus and after allowing time for neutralization to occur was injected intracerebrally into a monkey. Forty days later the animal was living and had shown no symptoms. At this time another test was made, using 1 c.c. of serum and 0.3 c.c. of virus. Eleven days later the animal's movements became slow, it grew thin and weak and died on the fourteenth day after the second injection. At autopsy the grey matter of the cord looked somewhat edematous. There were no definite microscopic lesions of poliomyelitis.

Experiment VI.—The serum for this experiment was obtained from M. S., two and one half years old, a patient in the hospital suffering from cerebral hemorrhage. Two tests were made with this serum; in one, 1 c.c. of serum was mixed with 0.1 c.c. of virus; and in the other, 1 c.c. of serum with 0.3 c.c. of virus. The first animal died of poliomyelitis in seven days and the second in eight days.

SERA OF SUSPECTED CASES.

Experiment VII.—The serum was obtained from Patrick T., aged twenty-three months, a patient in the poliomyelitis ward. At the time of admission to the ward, the patient's sister was suffering from poliomyelitis with paralyses. The patient on admission was drowsy, showed the characteristic irritability on being disturbed, and had slight fever. The spinal fluid showed 94 cells per c.mm., practically all of which were of the mononuclear type. Although the mother noticed some weakness in the child when he got up from a sitting posture, the child showed no paralyses while in the hospital. One c.c. of the serum obtained from this patient seven days after the onset of the disease, was mixed with 0.1 c.c. of active virus and injected intracerebrally into a monkey. Nine days later the animal died of typical poliomyelitis.

Experiment VIII.—The serum for this test was obtained from Donald P., the brother of a patient who died of poliomyelitis in the hospital ward. He had shown suspicious symptoms at the time of the other child's illness. The serum was obtained about three weeks after the suspected attack and 1 c.c. of it was mixed with 0.1 c.c. of virus and injected intracerebrally into a monkey. Ten days later the animal died, having developed a characteristic poliomyelitis.

Experiment IX.—The serum used in this test was obtained from H. B., a patient in the hospital. A constant playmate had died of poliomyelitis some two weeks before the patient's admission. The patient, when studied in the ward, had slight fever and showed marked drowsiness. There were no paralyses but slight spasticity on walking and some rigidity of the neck. The maximum cell count in the spinal fluid was 62 cells per c.mm., chiefly mononuclear. The globulin content of the fluid was slightly increased. A monkey, injected intracerebrally with a mixture of 0.1 c.c. of active virus and 1 c.c. of the patient's serum, obtained three weeks after the onset of the illness, showed no signs of poliomyelitis.

SERA OF TYPICAL CASES OF POLIOMYELITIS.

Experiment X.—For this experiment, serum was obtained from C. T., an adult who had suffered from poliomyelitis thirty years previously. Two tests

were made with this serum. In one, 0.1 c.c. of active virus was mixed with 1 c.c. of serum; and in the other, 0.3 c.c. of the virus was mixed with 1 c.c. of serum. Two monkeys were injected with the material in the usual way. Both animals were protected, neither showing any signs of poliomyelitis.

Experiment XI.—In this instance the serum was obtained from an individual, E. R., sixteen years of age, who had had poliomyelitis fourteen years previously. One c.c. of serum was mixed with 0.1 c.c. of active virus and injected into a monkey. Twenty days later the animal developed a paralysis of one leg. The paralysis, however, did not progress and the condition of the monkey returned to normal, with the exception of a residual paralysis. Inasmuch as the dose of virus given has invariably proven fatal in unprotected monkeys, the serum of this patient must be considered as having some protective power.

Experiment XII.—The serum for this test was obtained from C. A., a child eleven years old who had had poliomyelitis ten years previously. One c.c. of serum was mixed with 0.1 cc. of active virus and injected into a monkey in the usual way. The animal was protected, showing no symptoms of poliomyelitis at any time.

Experiment XIII.—The serum used for this experiment was obtained from M. G., four years old, a patient in the poliomyelitis ward. On admission there was partial paralysis of the external ocular muscles, high fever, and some stiffness of the neck. The maximum cell count in the spinal fluid was 320 cells per c.mm. One c.c. of serum obtained thirty days after the onset of the disease was mixed with 0.1 c.c. of active virus and injected intracerebrally into a monkey. Protection was complete, the animal showing no evidence of poliomyelitis.

The above series of tests of the ability of normal, of suspected, and of certain poliomyelitic sera to protect monkeys against infection with the virus of poliomyelitis, needs very little comment. Judging from the results obtained, the test cannot be considered to give specific evidence as to whether a given individual has or has not suffered from a previous attack of poliomyelitis. Of the six normal sera tested, but two failed to protect animals against infection, whereas four gave complete protection, and three of the latter protected against such large doses of the virus as 0.3 of a cubic centimeter. It is, however, fair to say that in two of the instances in which protection was obtained, the individuals had been constantly exposed to infection with the virus of poliomyelitis, and during the time of exposure had suffered from an indefinite illness which might possibly be attributed to an abortive attack of poliomyelitis. In three instances serum was obtained from persons suspected of having poliomyelitis. Of these sera, one protected a monkey against experimental infection and two failed to protect. Two of these individuals were carefully observed in the hospital

and both presented fairly definite clinical evidence of the disease. The serum of one of the latter protected a monkey against infection, and the serum of the one that failed to protect was obtained too early (seven days after the onset of the disease) for the immune bodies to be present in the serum in sufficient concentration. Four sera obtained from individuals who had had a characteristic poliomyelitis protected animals against fatal infection in every instance. One serum which was obtained thirty years after the occurrence of the disease, protected against both 0.1 of a cubic centimeter and 0.3 of a cubic centimeter of active virus. From these results it would seem that in typical instances of poliomyelitis, the serum of the infected individual probably always contains, after a certain interval, protective bodies, and that these substances persist for a very long time. In spite of the irregular results obtained with normal sera and the sera of suspected individuals, one cannot as yet rule out the specificity of the test. In the case of apparently normal persons whose serum protects, the possibility must always be borne in mind that he may previously have suffered from an unrecognized, abortive attack of the disease. The accurate determination of the protective value of normal serum can probably be made only by a study of sera from very young individuals in whom the possibility of previous infection can certainly be excluded.

PATHOLOGY.

The earliest pathological studies in poliomyelitis were made on chronic cases of the disease, and the lesions described were the atrophic scars found in the anterior horns of the cord. With the development of a better clinical knowledge of the acute stage of the disease, a new pathology arose which laid especial stress on the earliest morphological changes in the cord, and explained their relation to the development of the chronic forms. The work of many investigators, notably Harbitz and Scheel,[21] Wickman,[22] and Strauss,[23] has produced an accurate picture of the anatomical lesions

[21] Harbitz and Scheel, *loc. cit.*

[22] Wickman, Die akute Poliomyelitis, *loc cit.*, p. 13.

[23] Strauss, The Pathology of Acute Poliomyelitis, Report on the New York Epidemic of 1907 of the Collective Investigation Committee, New York, 1910.

occurring in the brain and spinal cord. The attention of observers has hitherto been almost wholly centered on the lesions of the central nervous system, and in consideration of the fact that the most striking disturbances, both pathological and clinical, are associated with the nervous system, this is not remarkable. In view, however, of the very definite and constant changes which are found at autopsy in other viscera, it is rather strange that they should have been almost wholly disregarded, and that so little emphasis should have been put on the fact that acute poliomyelitis is essentially a general infection. Rissler,[24] Strauss, Harbitz and Scheel, and Wickman have all noted the presence of lesions outside the nervous system, but have passed them by as having little bearing on the disease. As a matter of fact, the recognition of acute poliomyelitis as a general infection has an important bearing both in explaining the clinical course of the disease and as affecting any possible means of treating it.

Inasmuch as the lesions of the central nervous system are of chief importance, they may be considered first. At autopsy the meninges are usually found to be somewhat edematous and injected. There is little increase of cerebrospinal fluid. The brain and cord, on section, have a moist, translucent, edematous appearance, and the gray matter of the cord is often swollen so that it projects above the level of the white matter. It is darker than normally in color and is typically of a grayish pink hue. Not infrequently minute hemorrhages can be distinguished in the gray or white matter.

The exact path by which the virus enters the body is at present not definitely known, but there is clinical and experimental evidence which makes it seem probable that infection frequently gains access from the upper respiratory tract. It has been shown both anatomically and experimentally (Flexner[25]) that the upper nasal cavities are in direct communication with the meninges by means of the lymphatics which pass outward with the filaments of the olfactory nerve. The view that the virus may enter the body by means of those lymphatics and thus exert its first effect upon the meninges is

[24] Rissler, Zur Kenntniss der Veränderungen des Nervensystems bei Poliomyelitis anterior acuta, *Nord. med. Ark.*, 1888, xx, 1.

[25] Flexner, *Jour. Am. Med. Assn., loc. cit.*

strengthened by the anatomical findings. The earliest change which has been described in the nervous system is hyperemia and the collection of numbers of small mononuclear cells, probably lymphocytes, in the perivascular lymph spaces of the blood-vessels of the leptomeninges. These lymphatic spaces surrounding the vessels are anatomically processes of the arachnoid spaces, and the lymph in them is in communication with the cerebrospinal fluid. This first change, then, is an acute interstitial meningitis, which is not associated with fibrin formation or with exudate on the surface of the meninges. It is usually most marked on the anterior surface of the spinal cord, and especially in the anterior fissure, from which the larger vessels enter the cord (figure 1); but smaller collections of cells are often found along the meningeal vessels which are situated over the lateral and posterior aspects of the cord. The blood supply of the cord is derived from the vessels of the meninges, and with the advance of the pathological process, this perivascular infiltration follows along the vessels as they enter the cord from the meninges. Thus the earliest change that is found in the cord itself, both in human beings and in the experimentally produced disease, is hyperemia and the collection of small round cells in the lymph spaces surrounding the vessels. This cellular exudate forms a sheath apparently completely surrounding the vessels for long stretches (figure 2), and in many places the cells are so numerous that they form thick collars which seem to press on the lumen, and thus exert a mechanical effect in obstructing the circulation. While the cellular exudate is in the outer part of the vessel wall, it is probable that there is often some effect, either toxic or mechanical, on the intimal lining of the vessels, for hemorrhages (figure 3), minute or extensive, are frequent findings, and one of the prominent features of most cases is the extensive edema. These three factors, cellular exudate, hemorrhages, and edema, all of them dependent on vascular changes, may perhaps be regarded as the primary reaction of the nervous system to the virus of poliomyelitis. The effects produced on the nerve cells themselves are probably either dependent on these vascular disturbances or they may be due to a direct action of the virus. This superior importance of the vascular system in determining the nervous lesions has for a

long time explained the fact that the cervical and lumbar enlargements of the cord are most affected, and that the anterior horns of the gray matter are more involved than the posterior horns or the white matter. These are, of course, the regions of the spinal cord to which the blood supply is most abundant. Moreover, the frequency with which lesions are asymmetrical probably depends on the irregularity with which the vessels supplying the cord are given off at different levels. It has been suggested that the process by means of which the vascular lesions affect the nerve cells may be essentially a mechanical one. It is quite impossible to exclude the fact that the virus may exert some directly toxic action on these cells, but in many ways, the clinical and anatomical pictures are readily explained by the presence of the circulatory disturbance and of the exudate. On such an hypothesis the damaging effects can be assumed to result in part from the direct pressure on the nerve cells of hemorrhages, edema, and exudate. There is also the additional factor of anemia following the constriction of the blood vessels by the same mechanism. On account of this pressure and anemia, the nerve cells degenerate. If the hemorrhage and exudate are absorbed soon enough, the cells may recover their function. If, on the other hand, the anemia and pressure have been prolonged or excessive, the nerve cells go on to complete necrosis. Histological examination shows nerve cells in all stages of degeneration, from those with the slightest changes in their protoplasm to others of which only a granular detritus remains. A most striking picture is formed by the entrance of polymorphonuclear neurophages into the necrotic nerve cells (figure 4). A single nerve cell may be invaded by a dozen of these phagocytes, and by means of them necrotic material is completely disposed of. In more severe lesions, one sees the hyperemia, the perivascular infiltration, hemorrhages, edema, and a diffuse cellular infiltration throughout the gray and white matter, but nerve cells may be completely absent from the picture. These changes, most prominent in the anterior horns of the gray matter, are not sharply circumscribed, but are scattered more or less diffusely through both the gray and white matter of the cord (figures 5, 6, and 7).

The same sequence of changes, vascular disturbance, and subse-

quent degeneration of the nervous elements, is found to a less degree in the brain, medulla (figure 8), and pons. Hyperemia and a moderate amount of cellular infiltration may be found in association with the vessels of the cerebrum and cerebellum, but lesions extensive enough to produce motor symptoms are exceedingly rare. The medulla and pons show some slight degree of involvement in most cases, and one frequently finds in them a marked cellular exudate and many hemorrhages. It is noteworthy, however, that it is often extremely difficult to reconcile the clinical symptoms, which are referable to pontine lesions, with the actual autopsy findings. Cases which have shown bulbar paralyses in life may fail to show adequate anatomical lesions to account for them, and other cases which have given clinical evidence of spinal involvement only may show changes through the pons and medulla.

Of practically constant occurrence are the lesions in the posterior root ganglia. The histological changes are similar to those that take place in the cord itself (figures 9 and 10). There is an infiltration of small round cells in the lymphatic spaces surrounding the vessels which enter the ganglia from the meninges. This has been shown experimentally to be the first step in the process. Then follows a more general, diffuse exudation of cells, degeneration and necrosis of the nerve cells, and finally the entrance of polymorphonuclear leucocytes into the necrotic cells and removal of the disintegrating cells by neurophages (figure 11). The suggestion has been made that these lesions in the sensory ganglia may in part account for the pain which is such a constant feature of the acute stage of the disease. Another element in the production of pain is the cellular infiltration which is found along the nerve roots.

The changes which are found in other organs in acute poliomyelitis are less striking than those in the nervous system, but they have been, in our experience, practically as constant. In all of the eleven acute cases which we have been able to examine, there has been more or less extensive involvement of the lymphoid tissue and of parenchymatous organs. The lymphoid tissue throughout the body appears to react to the virus. The Peyer's patches of the intestine and the mesenteric lymph glands show perhaps the most marked acute swelling. The mucosa over the Peyer's patches is,

however, unaffected. There is also definite, and sometimes pronounced enlargement of the substernal, bronchial, cervical, axillary, and inguinal lymph glands and of the tonsils. The spleen is frequently somewhat enlarged, and on section the Malpighian corpuscles stand up in raised, pale, obviously translucent nodules. The thymus shows changes identical with those in the lymphoid tissue elsewhere. On histological examination, one is struck by the resemblance of the lesions found to those described by Mallory[26] in typhoid fever. The reaction is, in general, the same throughout the lymphoid tissue, regardless of its location. On histological examination some of the lymphoid nodules may present a normal appearance, but the majority consist of a zone of lymphocytes surrounding a more or less sharply circumscribed pale center (figure 12). High magnification shows the center of the lobule to consist chiefly of large endothelial cells with oval vesicular nuclei. These cells are similar to the cells lining the lymph sinuses, but most of them are larger, more swollen, and take the stain very lightly. Sometimes the nuclei look like pale shadows, and the outline of the protoplasm is so faint that it can scarcely be distinguished. Where they are closely packed, the individual cells appear to be fused together to form compact masses. The better preserved of these cells are markedly phagocytic and frequently contain many particles of necrotic cells. These cell inclusions are surrounded by a lightly stained halo and are apparently situated in vacuoles in the protoplasm. Scattered throughout the center of the nodule are many broken down cells and granular fragments of necrotic nuclei. The cells which are going to pieces are for the most part lymphocytes, but the endothelial cells also seem to swell up and finally disintegrate. In areas with extensive necrosis there is often an invasion by polymorphonuclear leucocytes.

In the lymph sinuses there are also large numbers of the same phagocytic endothelial cells. Many of them are of great size and contain necrotic fragments of nuclei, whole lymphocytes, or numbers of red blood corpuscles. In the lymph sinuses, there is extensive proliferation of the endothelial cells, as is evidenced by the frequency with which mitotic figures are found. Numbers of ne-

[26] Mallory, *Jour. Exper. Med.*, 1898, iii, 611.

crotic cells are met with in the lymph sinuses, but, in general, necrosis is a more prominent feature in the centers of the lymphatic nodules, and proliferation in the lymph sinuses.

Among the parenchymatous organs, "cloudy swelling," such as has been frequently described, is usually met with. In the liver, however, there are other more striking changes, and these again resemble in character those that have been described by Mallory and others in typhoid fever. They are for the most part sharply circumscribed areas, ranging in size from lesions which consist of one or two cells to others which include nearly one eighth of a liver lobule, in which degeneration of liver cells and infiltration of lymphoid cells and polymorphonuclear cells have taken place (figure 13). The number of these necroses is very variable. They may be rather difficult to find, or there may be many of them in a single low power field. The lesions are apparently closely associated with the blood vessels, and while chiefly with the portal vein, they are also associated with central or sublobular vessels. The section is, however, sometimes cut so that the relation to the blood-vessels is not directly evident. The lesions are round or oval in cross section, but there may be long finger-like projections, extending out from the main area, and involving one or two columns of liver cells. It is striking that the liver cells directly adjacent to the infiltrated areas are usually completely spared, but occasionally one finds that some of them show a homogeneous quality of the protoplasm, and an exaggerated affinity for eosin, which suggest a beginning involvement through a peripheral spread of the lesion.

The character of the lesion varies, of course, with its size and age, but in general it is very constant. In the earlier lesions one finds one or two disintegrating liver cells, with homogeneous, hyaline, pink-staining protoplasm, and occasionally with irregular, distorted nuclei. In the older lesions, even more apparent than the necrotic liver cells is the evidence of an early and rapid proliferation. Scattered through practically all the lesions are small groups of liver cell nuclei, frequently a nest of three or four nuclei surrounded by infiltrated tissue, or several nuclei apparently beginning to proliferate within a degenerated liver cell body. The protoplasm around these dividing nuclei may appear as a faint pink halo, or it may be

quite impossible to make out any protoplasm at all. In older lesions, the protoplasm is better defined and the cells have frequently arranged themselves circularly, in acini, or in double columns. This arrangement of cells simulates that in normal bile ducts, but it is found in association with nearly all the lesions, irrespective of their position in the lobule, although most frequently adjacent to the portal spaces. According to the position of the infiltrative lesion, much or little fibrillated tissue appears among the degenerating and infiltrating cells. The greater amount of fibrillated tissue occurs in and adjacent to the portal spaces, where all the lesions of an early cirrhosis may be displayed. That this condition is of the nature of a beginning cirrhosis is further indicated by the microscopic depressions of the capsule of the organ, where the lesions extend toward and reach that structure. However, the process probably is not permanent, since it is so young and cellular that it can readily undergo retrogression; while the infiltrated nodules elsewhere in the lobules are surely easily subject to restoration. One case has indeed come to autopsy, the child dying of a laryngeal diphtheria two months after the onset of poliomyelitic paralysis affecting both legs. In the liver of this case were remains of the portal infiltrative lesions described and, in addition, younger necrotic and infiltrated lesions among the columns of liver cells. The former probably had been greater and were diminishing; it is uncertain whether the latter were residues of the poliomyelitic infection or the result of the recent diphtheritic process. That these changes in the lymphoid tissues and in the liver are, in fact, a part of the reaction of the body to the virus of poliomyelitis, would seem to be made certain by the fact that exactly similar lesions may be found in the organs of monkeys which have been experimentally infected with the disease (Flexner[27]).

The demonstration of such a widespread reaction to the virus is wholly in line with recent clinical and epidemiological advances which tend to recognize acute poliomyelitis as a general infection. The disease must be regarded as a generalized process which affects parenchymatous organs, lymphoid tissue, and more especially the nervous system. It is possible that two distinct effects of the disease

[27] Flexner, *Folio serolog.*, 1911, vii, 1101.

on the organism should be differentiated. On the one hand is the general toxemic process which affects organs throughout the body, but which apparently acts mildly. On the other hand is the local process in the spinal cord, producing death by destruction of the nerve cells controlling respiration. The anatomical findings which point to an action of the virus of poliomyelitis on the lymphoid tissue throughout the body may well be correlated with the results obtained by the inoculation of emulsions of lymphoid organs into monkeys. Not long after the disease was first transferred to monkeys by the intracerebral inoculation of filtrates of the spinal cord, the virus was proved, in the same manner, to be present in a mesenteric lymph gland. Since then similar positive results have been obtained with still other lymphatic glands in the monkey, and with emulsions of the tonsils in man and the monkey.

These observations are not, however, of the same significance and importance. What they tend to indicate is that the living virus may come to rest for a time in organs outside the central nervous system, to which it is conveyed by the blood. It does not establish the fact that the visceral lesions outside the nervous system are caused directly by the virus, rather than by some secondary toxic substance produced in the course of its proliferation. Since the only means at present available to demonstrate the presence of the virus is the inoculation of monkeys, the tests made are too few to determine how widespread in the organs the virus really is. It is established that it is present in the central nervous system, even when it cannot be demonstrated in the viscera generally. But the finding of the virus in the tonsils and nasal mucosa with as great constancy as in the nervous system indicates that these organs play a part in the conveyance of the virus into and away from the central nervous system. The virus is regularly present in them in fatal and doubtless in non-fatal cases of poliomyelitis in children, and it has been demonstrated in the nasal mucosa, the tonsils, and even in the nasal mucous membrane in infected monkeys; it finds its way into the nasal mucous membrane, even when injected into the peritoneal cavity. The evidence, therefore, is strong that, as was first pointed out by Flexner, the upper respiratory mucous membrane provides for both the ingress and egress of the virus of the disease, through which infec-

tion is produced and the renewal of the cause of the disease maintained.

SYMPTOMATOLOGY.

CLINICAL CLASSIFICATION OF CASES.

The study of any disease is simplified if the various clinical forms in which it may appear can be grouped together into a few definite types. While no two cases of any disease are ever exactly similar, one probably finds variety of gross clinical manifestation most strikingly illustrated in the diseases of the nervous system, and a pathological process which may extend more or less continuously through brain, medulla, pons, spinal cord, and spinal ganglia, or which may be localized in any part of this system may produce signs and symptoms of very diverse character. Such are the possibilities that are present in poliomyelitis, and it is no wonder that the clinical classification of cases has been the subject of much discussion. The most generally accepted system has been that of Wickman, who recognizes eight forms: (1) the spinal, poliomyelitic form, (2) the cases simulating Landry's paralysis, (3) the bulbar or pontine form, (4) the encephalitic form, (5) the ataxic form, (6) the neuritic form, (7) the meningeal form, and (8) the abortive cases. While such a classification certainly covers all possible cases and makes it easy to place most cases in their appropriate groups, it does not appear to us to be wholly satisfactory. It is based neither on pathological anatomy, nor on clinical symptomatology, but on a mixture of the two. The spinal, the bulbar, and the encephalitic forms are anatomical forms; the neuritic, the meningeal, and the ataxic forms are essentially symptomatic forms. A more satisfactory classification would be either anatomical or clinical. Such a classification would simplify a confusion arising from the use of two systems, for it is impossible to draw any hard and fast line between some of the groups. Thus most neuritic forms are essentially of the spinal type, and most meningeal cases are either spinal or bulbar. The ataxic form Zappert regards as based on a single, not especially prominent symptom, rather than on an anatomical foundation. The cases simulating Landry's paralysis are in the main instances of the spinal type in which the process advances, usually to end in death

from involvement of the respiratory muscles. Cases that are classed among the meningeal or neuritic forms are so classed because they show some of the clinical manifestations of meningitis or of neuritis, rather than because they have a pathology which is essentially different from other bulbar or spinal cases. These types of the disease are, in fact, not true types at all. They merely represent some of the variations in symptomatology which may be found more or less developed in association with either the abortive, the bulbar, the spinal, or the cerebral types of the disease, and their enumeration as definite forms is complicating and at times confusing. As a result of this duplicating and overlapping of subdivisions, the picture of the disease, as a whole, is made much more complicated and obscure than is necessary.

More simple and, at the same time, more practical is the classification proposed by Müller. Adopting, as far as possible, an anatomical basis, he proposes the four following classes: (1) the spinal form, (2) the bulbar form, (3) the cerebral form, and (4) the abortive cases. In any system of classification, the abortive cases must, of course, form a distinct type. In considering the cases, however, which result in paralysis, one would seem to get a clearer outline of the disease process, if the cases are looked at from the point of view of pathological physiology and divided into two main groups: (1) those cases in which the upper motor neurone is primarily affected, and (2) the larger group of cases in which the lower motor neurone is involved. The first group is the cerebral form of Müller, and the second group is composed of his spinal and bulbar forms. The two latter forms it is wiser to consider together, as in both the lesion is essentially the same, a lower motor neurone lesion involving, on the one hand, the nuclei of the pons or medulla and, on the other hand, the anterior horn cells, the result being in either instance a flaccid paralysis with subsequent atrophy. The two types which are thus based on similar pathological lesions are also inseparable clinically, for a somewhat larger number of cases occurs with cranial nerve involvement, in association with spinal lesions, than occurs with cranial nerve involvement alone. The third group of cases in Müller's classification is the cerebral type, the same group to which Wickman gives the name encephalitic. This

is a fairly definite pathological entity, at least in so far as the most prominent lesion apparently involves the upper motor neurone. While the bulbospinal type is characterized by flaccid paralyses, this cerebral type is characterized by spastic paralyses or by other evidence of a lesion above the lower neurone. The exact location of the pathological processes in these cases is not wholly clear, but it seems probable that the upper neurone may be attacked in either or both of two places. Some few cases are probably due to lesions in the cortex with involvement of much larger areas than are commonly found. Other cases, the majority, seem to depend on involvement of the pyramidal tracts, either high up or in the cord. That this class of case, originally described by Strümpell as polioencephalitis acuta is very uncommon, but that it is in reality a form of infantile paralysis, has been made quite certain by epidemiological studies which show the spastic type occurring in the same epidemic and in the same house with the flaccid types, by the occasional occurrence of both spastic and flaccid paralyses in the same patient, as well as by the pathological studies of Harbitz and Scheel.

It would seem, then, that the best appreciation of acute poliomyelitis, from the clinical point of view, is obtained if one recognizes three groups of cases. The first group consists of the *abortive cases,* cases of infection which never become paralyzed. The second or *cerebral group* contains the rare cases in which involvement of the upper motor neurone with resulting spastic paralysis is the chief characteristic. The third or *bulbospinal group* is much larger and comprises all cases with lesions in the lower motor neurone and flaccid paralyses.

Such a classification is, of course, open to the objection that many cases are not purely of one type, either anatomically or clinically. Thus most bulbospinal cases show some pathological foci in the brain, and cerebral cases may show lesions extending into the cord (Harbitz and Scheel). Then from the clinical point of view a certain number of bulbospinal cases develop symptoms as, for instance, ataxia, which may well depend on an upper motor neurone lesion, either in the brain or cerebellum, in the pyramidal tracts, or in Clarke's columns. When one recognizes how diffusely the pathological process extends through the whole central nervous system,

it becomes evident that it is impossible to make any hard and fast classification which shall be strictly applicable to all cases. The best one can do is to attempt to reconcile the chief clinical symptoms with the predominant anatomical lesion. In the following sections, we shall consider the symptomatology and course of each of these types in detail.

PREDISPOSING CAUSES.

Acute poliomyelitis is a disease which shows very definite seasonal variations in its incidence. The records of epidemics in many countries show that it occurs during the summer and reaches its maximum in the late summer and early autumn. In the Swedish epidemic of 1905, 86 per cent. of the cases had their onset between July and October, and 35 per cent. during August. The New York epidemic of 1907 showed a very similar curve, reaching its maximum, however, in September. Other epidemics show some slight variations in duration and in the period at which the highest point is reached, but that the disease is distinctly one of summer and fall has been generally accepted. There are a few well substantiated epidemics which prove that poliomyelitis may also occur during cold weather. Wickman cites one epidemic in the north of Sweden, lasting through the winter, and reaching its maximum in April and May. He also reports three closely related epidemics, occurring in neighboring sections of the country, the first (eighteen cases) lasted from June to October, the second (twenty-seven cases), from July to December, and the third (sixty-two cases), from the end of September to February, with its maximum in November and December. These winter epidemics are of interest in relation to attempts that are being made to account for the spread of the disease by some intermediary insect host. Stress has been laid on the frequent occurrence of epidemics of poliomyelitis in unusually hot, dry years, but this relationship is certainly far from constant.

It has been generally noticed that acute poliomyelitis is a disease of open country, rather than of cities. Among our own cases, a relatively larger number of children came from the suburbs and surrounding country than from the densely populated tenement district in which the hospital stands. Moreover, a considerable

proportion came from families which are in comparatively well-to-do circumstances and in which the children enjoy every comfort and care. Pneumonia, measles, and other acute diseases are often mentioned as predisposing causes of poliomyelitis, but in our experience the absence of any history of previous illness has been much more noticeable. In general, they had been perfectly healthy children.

Childhood is essentially the age which is most susceptible to poliomyelitis, but that adults are by no means immune is shown by Wickman's statistics. More than one fifth of his cases were persons over fifteen years old. He mentions one case in a man of forty-six years, and instances of the disease occurring in even older persons are on record. In the New York epidemic of 1907, the youngest case was two weeks old. Three cases seen by us were three months old, and fifteen cases were between six and twelve months. As in the 1907 epidemic in New York, the majority of our cases were between the ages of one and three years. The figures obtained by Müller agree rather remarkably with ours in respect to age incidence. Of his cases 96 per cent., and of ours 97 per cent. were in the first decade, while 90 per cent. of his, and 89 per cent. of ours were below the age of five. Perhaps the age most liable to infection is the latter half of the second year. Both sexes are almost equally susceptible to the disease, the number of males being slightly greater than the number of females.

TABLE OF AGE INCIDENCE.

	Months.		Years.													Total.
	Under 6	6–12	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–15	15–20	20–25	Over 25	
Rockefeller Institute Hospital, 1911..	3	15	62	29	18	13	6	4	0	2	1	3	0	1	0	157
New York Epidemic, 1907.....	62		221	180	106	63	28	18	11	11	7	14	5	1	2	729
Wickman, Sweden, 1905.....	183				214			179			229			220		1,025

THE PRODROMAL PERIOD.

In most typical cases of infantile paralysis, the course of the disease is fairly constant. A period of incubation is followed first by a period of prodromal symptoms, then by an acute stage with paralysis, and finally by a stage of retrogression. The duration of the incubation period is variable. In the experimental disease in monkeys, Flexner has found that the time elapsing between inoculation and the onset of paralysis is from three or four days to thirty-three days, the average being eight or nine days. Prodromal symptoms are of short duration in the monkey, rarely lasting over twenty-four hours. With a virus that has been passed through several generations of monkeys, and has thus become adapted to its new host, the period of incubation is much more constant than it is on the first transfer from man. Such a fixed virus causes paralysis with great regularity in seven or eight days. In the disease in man it is obviously much more difficult to determine the exact length of the incubation period. Thus, even when two cases appear in one family, it is often impossible to be certain at what time the second case became infected by the first, or even whether both were not infected at the same time from a common source. Wickman considers the incubation period as being from one to four days, his opinion being based on the interval between the dates of onset of the disease in two persons of the same family. Our own observations of families in which two cases have occurred would lead us to a nearly similar conclusion, but we believe that these are in all probability simultaneous infections from a common source. At any rate, previous contact had been so intimate that infection might have taken place at any time during the incubation period of the first case. In one family at least, two children became paralyzed on the same day. Müller has seen six instances in which the length of the incubation period could be determined more accurately, and he concluded that it varies between five and ten days. The average incubation period was about a week, thus practically the same as is found in the experimentally produced disease.

Following the incubation period and preceding the onset of paralysis, there is, in the vast majority of cases, a period marked by prodromal symptoms. These symptoms are at times of such

a mild and fleeting nature that they may be entirely overlooked, but except in the case of small babies, and with unusually unintelligent parents, careful history taking will almost always bring out evidence of some prodromata. There is, however, a small number of cases in which the acute stage with paralysis really seems to be the first outward manifestation of the disease. We have seen several cases occurring in intelligent families in which, in spite of careful questioning, it was impossible to obtain evidence of prodromal symptoms. The usual history in this rather exceptional type of onset is that the child goes to bed perfectly well and wakes up paralyzed. These represent but a very small fraction of the total number of cases; the others give a definite history of a prodromal stage. The prodromal period, which, until recently, received little attention at the hands of clinicians, has, in the light of our present knowledge of epidemiology and of our hopes for a therapeutic control of the disease, assumed an unexpected prominence. It is during the prodromal period that one must isolate and quarantine, if such measures are expected to be efficacious, and it is only in the prodromal period, before an extensive destruction of nerve cells takes place, that one can ever hope to make treatment efficient. This is, therefore, the most important stage in the course of the disease, for on its recognition depends the possibility of controlling the infection.

The duration of the prodromal symptoms is variable. In our series of cases they lasted, in most instances, from one to seven days, with one to three days as an average. The usual history is that the symptoms increase progressively in severity through the prodromal period, but occasionally, after being sick for two or three days, there is a distinct cessation of symptoms and the child apparently recovers. One or two days later, however, and perhaps without further warning, the child becomes paralyzed (case 1, page 122).

The severity of the prodromal symptoms, also, varies greatly from case to case. While in one child they are so mild and transient that they are quite disregarded, in another they may be alarming. In a considerable percentage of our cases the children were sick enough during the prodromal period to make the mothers feel that a doctor should be sent for. It is a generally accepted fact that the severity of the prodromata bear no relation to the extent of the ensuing paralysis or to the subsequent course of the disease.

The prodromal symptoms are, on the whole, of a general nature. A few are more specific in character and shed some light on the nature of the disease. For the most part they are such as may appear at the onset of almost any acute infection or, indeed, such as occur in children with very little recognizable cause. While individual cases show considerable differences, there are certain groups of symptoms which are present in the majority of all cases. Other types of symptoms have been shown to have a tendency to vary from epidemic to epidemic. Thus in the epidemic which occurred in Hesse-Nassau and was reported by Müller, the majority of the cases had prodromal symptoms referable to the respiratory tract. In the neighboring province of Westphalia, during the same epidemic, Krause[28] found a preponderance of cases with gastro-intestinal symptoms. Two thirds of his cases had a marked diarrhea. During the New York epidemic of 1907, intestinal symptoms were not marked, but gastric disturbances were common.

Probably the most constant feature of the prodromal period is *fever.* In most cases this is noted among the earliest symptoms, in fact the usual story is that the patient was perfectly well in the morning, but towards evening became feverish. Sometimes fever was apparently not noticed until the second or third day after the child became sick, but this may be due to the fact that a history of the presence or absence of fever is usually based on the appearance of the child and the temperature of the skin, rather than on actual temperature determinations with the thermometer. However, in a number of cases, actual measurements showed that a temperature of 103° F. or higher is of frequent occurrence. Onset with chill is comparatively rare. The febrile reaction generally lasts through the prodromal period, with slight morning remissions, and the temperature comes down to normal a few days after paralysis sets in. Occasionally the fever remits earlier, especially in those cases with a distinct interval, in which the child is without symptoms, between the prodromata and the acute stage.

Müller has laid special stress on profuse *sweating,* which he designates as one of the cardinal symptoms of the early stage of the disease. It was present in 75 per cent. of his cases. A definite

[28] Krause, *Deutsch. med. Wchnschr.,* 1909, xxxv, 1822.

explanation of the mechanism of its production he is unable to give, but he suggests that a pathological involvement of spinal sweat centers may play a part. The question of sweating was carefully gone into in our histories, but in only 25 per cent. of the cases had it been noticed at all. The number of cases with profuse, drenching sweats was very small. One naturally expects to find sweating a rather common accompaniment of fever, especially in children, and the comparatively slight transient sweats met with in most of our cases were no more than could be explained by the febrile reaction, and by the warm summer weather. On the whole, sweating was not a prominent symptom in this series of cases. The few cases in which sweating persisted after the temperature became normal will be discussed in the following section.

Associated with fever and perhaps dependent on it, is *drowsiness,* which was a very noticeable symptom in many cases. The children are apathetic and want to sleep most of the time. This drowsiness sometimes lasts only a day or two and then disappears, but often it increases and runs into a mildly stuporous condition with the onset of the acute stage. With flushed face and dulled, apathetic sensorium, the child may have a definitely typhoidal appearance. Frequently, the degree of apathy is out of all proportion to the height of the temperature. On being awakened, the patient is usually irritable. He cries and wants to be let alone to doze off again. Not infrequently drowsiness is less prominent, and *irritability* with a nervous, excited, complaining disposition replaces it. One baby, whom we watched in the prodromal period, was wide-eyed, alert, nervous, observant of all that went on around her, breathing rapidly, and crying when anyone approached her or attempted to touch her. This irritability is closely connected with what Müller considers as one of the three cardinal symptoms of the prodromal period, *hyperesthesia.* This symptom, which was present in three quarters of the cases in the Hesse-Nassau epidemic, was also very constantly seen in our cases. Müller mentions a hyperesthesia of the skin of which we found no instance in the preparalytic stage, but *pain on passive motion* was very frequently noticed. The mother would often say that the child cried when it was picked up, or when it was moved to change the bed linen. The movements which tend to produce pain

are essentially those which cause an anterior flexion of the spine; thus, flexion of the legs at the hip joint and flexion of the neck. At this early stage in the disease, retraction of the head is rarely met with, but stiffness of the neck and a definite resistance to flexion are very common. Similarly, the attempt to test for the Kernig sign often causes pain and evokes a resistant contracture of the hamstring muscles. This type of pain would seem to depend on the inflammatory involvement of the meninges, which has been shown in monkeys to occur in the preparalytic stage.

The frequency with which *spontaneous pain* occurs is difficult to determine on account of the youth of most of the patients. Those that are old enough, however, frequently complain of it. Pain in the head, in the back of the neck, in the back, or in the legs, is often present, though usually of a transient nature. Not infrequently there is pain in those limbs which subsequently become paralyzed. It seems probable that such pain may be due to the inflammatory reaction in the intervertebral ganglia. Pain, both spontaneous and that produced by motion, tends to increase as the acute stage is approached.

Another not uncommon forerunner of paralysis is *weakness* of one or more limbs or groups of muscles. A child may be noticed to "favor" one arm more than the other, or he walks with a limp, or his legs "give way under him." Physical examination shows no paralysis, but only a more or less definite weakness of the part. Associated with this may be changes in the tendon reflexes. These changes are by no means constant and may consist either in an exaggeration, in a reduction, or in complete loss. Perhaps most cases show first an early exaggeration of reflexes during the "irritative" stage, followed by a loss of reflexes just before the onset of paralysis. Less common "irritative" phenomena are *muscular twitchings* and tremors, such as were present in several of our cases. As one mother put it, "the baby's leg quivered." In the same category belong the general *convulsions,* which are, in our experience, rather unusual prodromata. Occasionally, however, they play a prominent part in the early history of the illness and suggest a cerebral disturbance.

In some epidemics of poliomyelitis, the disease is frequently ushered in by symptoms involving the respiratory tract. This, as has been pointed out, was notably the case in the Hesse-Nassau epidemic of 1909, in which over 50 per cent. of the patients had such symptoms. The character of the symptoms varied from a persistent cold in the head or a conjunctivitis, to an angina, bronchitis, or even a bronchopneumonia. While this type of symptom has not been at all frequent in our cases, we have had a few which gave a history of nasal discharge, and others of a more or less troublesome cough in the prodromal period. Occasionally, one of the older patients has complained of sore throat. The tonsils, as we have seen them, usually, however, after the onset of the acute stage, have been larger than normally and often ragged. Reddening of the tonsils has been rare, and in no case was a true exudate present. On the whole, disturbances of the respiratory tract have been rather remarkable for their absence.

Gastric symptoms, on the other hand, have been very frequently observed in our cases, as they were during the New York epidemic of 1907. Loss of appetite and nausea were fairly constant symptoms, and vomiting occurred in a large percentage of the cases. The vomiting was usually an early symptom and was in many instances the first evidence of sickness. The great majority of the children vomited only once or at most twice, and frequently it was noted that the vomiting followed immediately the taking of food. Many writers have called attention to the difference between this type and the recurring, projectile vomiting which occurs early in meningitis. Rarely, however, is persistent vomiting met with.

In the Westphalian epidemic described by Krause, over two thirds of the cases had a diarrhea in the prodromal period. Apparently it is more common to have a moderate grade of constipation. Very few of our cases showed any tendency to diarrhea. In almost all, the bowel movements had been normal, or there had been a degree of constipation such as one usually finds when a patient is put to bed. There has been some attempt to associate diarrhea with the pathological lesions of the intestine found at autopsy. Our experience makes such a relationship seem improbable, for all our autop-

sies showed well marked intestinal lesions, but in none of the cases had there been any diarrhea.

The clinical picture of the prodromal period of infantile paralysis shows very little that is of specific diagnostic value. In general, the history is that of a previously healthy child taken suddenly sick during the summer or early fall with fever, a moderate gastro-intestinal disturbance, a sore throat, or a slight cough, pain in the head, back, or legs, and often becoming quickly very drowsy. On physical examination there is stiffness of the neck with resistance to flexion; the Kernig manipulation of the legs and other passive motions are painful; the child is irritable when handled and wants to be left alone. Hyperesthesia is the most characteristic symptom, if one exists, but obviously there is little on which to base a diagnosis unless the relationship is close to other cases in an epidemic. The vagueness of the clinical picture is illustrated by a glance at the diagnosis made in some of our cases before the onset of paralysis: teething, tonsillitis, malaria, gastro-intestinal disturbance, and pneumonia. These show how far at sea one can be in the face of these prodromata. Have we, then, other methods of diagnosis that may be summoned to give assistance?

Müller has laid great emphasis on the fact that the blood picture is of value in the differential diagnosis of infantile paralysis. Both in the experimentally produced disease in monkeys and in the disease in man, he was struck with the tendency towards a leukopenia in the febrile stage. In his examination of the blood in fifteen cases, he found a low leukocyte count (3,000–5,000) in the majority, and a normal count in the remainder. Associated with this was a relative lymphocytosis. What proportion of these cases were in the preparalytic period and what proportion were in the early acute stage with a persisting fever, he does not state. These results, however, were not confirmed by La Fetra,[29] who found, in what Müller terms an "atypical epidemic" in New York, leukocyte counts running from 13,400 to 20,600. In four cases between the second and fifth day after the acute onset, all "still in the hypersensitive state with commencing paralysis," Gay and Lucas found leukocyte counts of 17,400, 10,000, 12,000, and 13,400. Our own experience with

[29] La Fetra, *Arch. Pediat.*, 1909, xxvi, 328.

blood examinations during the prodromal period has been limited, but those cases which we have seen lead us to believe that leukopenia is not a sign to be relied on. One case gave on the fourth day after the onset a white count of 7,000, and three days later, on the day preceding the paralysis, a count of 3,000. The other cases seen in the preparalytic stage had white counts of 8,800, 17,000, 15,000, 17,600, 15,000 and 35,000. The last was in a baby seven months old.

Of much more importance have been the results obtained by examination of the spinal fluid. In six instances we have done lumbar puncture in the prodromal period, and in all of them we have obtained pathological fluids. The character of these fluids will be discussed in detail in a later section; but it may be said that the general change consists in a moderate increase in pressure, an often striking increase in the number of cells per cubic millimeter, and in some cases an increase in the globulin content. The type of cells present may be either mononuclear or polynuclear. Pathological fluids at this stage of the disease have also been observed by Frissell (one case) and Lucas (nine cases). While the spinal fluid shows, according to our present knowledge, no changes that are specific for poliomyelitis, it often gives evidence very suggestive of meningeal involvement, and the absence of bacteria that are associated with the usual types of meningitis clears the field of many confusing elements. It would seem then that where one can correlate the clinical picture with the findings in the spinal fluid, a correct diagnosis should be frequently made in the prodromal period of poliomyelitis.

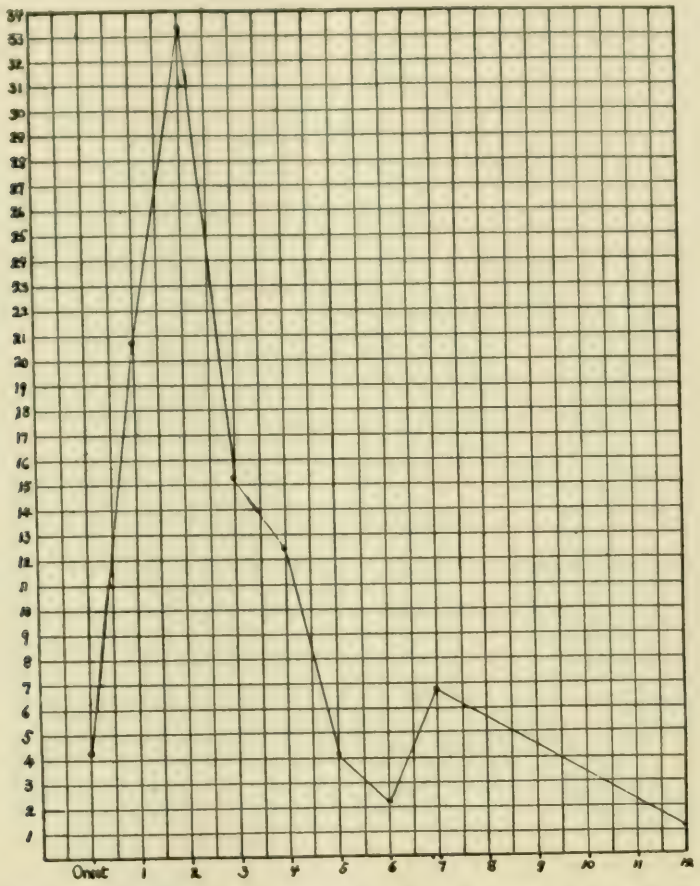
BULBOSPINAL TYPE.

ACUTE STAGE.

It is impossible to draw very definite lines about the stage of the disease to be called acute. The temperature is not a definite criterion of the stage of the pathological process, for it is often gone before paralysis appears. Frequently, also, a child may become more prostrated and more stuporous several days after the temperature has fallen to normal. A better though still somewhat unsatisfactory signal of the fully developed disease is the *advent of*

paralysis. Any limits, therefore, put upon an acute stage in poliomyelitis are arbitrary and necessarily loose; but we have considered the acute stage to include the days between the onset of the paralysis and the disappearance of tenderness on spinal flexion.

The clinical picture of the fastigium of acute poliomyelitis, like that of the period of onset, is as varied as its pathological mechanism would indicate. It is convenient, however, to describe this part of



TEXT-FIG. 1. Chart showing the relation of the onset of paralysis to the day of disease.

the disease under four groupings: (I) the usual form; (II) the cases which have no prodromata and whose first symptom is paralysis; (III) the cases with a remission of symptoms and delayed paralysis; and (IV) the cases with deep stupor.

I. Usual Form.—In the commonest form of the disease, the paralysis appears on the first or second day after onset (text-figure 1). At this time the child may be found lying on its back,

with thighs slightly flexed and everted in a frog-like manner, and the head usually rotated to one side. The eyes are partly or wholly closed and there is a peculiar tired, wilted expression. Not infrequently, the chin is pointed upward a little, indicating a small degree of retraction. From this drowsy or almost sleeping condition, the child can be roused suddenly, often by the gentlest touch or manipulation of an extremity. Very frequently when the leg is lifted only a few inches from the bed, an expression of annoyance, rather than distress, crosses the face, and if the leg be the paralyzed one, the child often tries to free it from the examiner's hands by twisting the trunk and shoulders. This procedure is a surprisingly common one and is usually accompanied by a pettish, fretful, rather bored look and whine. But when the examiner stands back from the bed, the patient lapses almost at once into the drowsy state. In contrast to these cases are those of a more sthenic nature with evidently greater meningeal irritation. These children are almost always found lying on their sides with their heads drawn well back and their knees and thighs flexed, a typical meningitis posture. Occasionally a true opisthotonos appears. One such patient could not be made to lie on his back, evidently because the flat line of the mattress prevented the slight degree of opisthotonos which made him comfortable; but when a pillow was doubled up under the lower dorsal and lumbar region so that an opisthotonos was induced by gravity acting on hips and shoulders, the child promptly went to sleep in the dorsal position. Such cases also are apt to have the drowsy, wilted look. Much more rarely, the child is wide-eyed and has an anxious, apprehensive, rather frightened expression. Many times these patients do not wait to be touched before objecting, but cry out even when the nurse or doctor approaches the bed; and it is surprising how keenly the little patients seem to determine whether or not an approaching person is coming to perform some service which necessitates manipulation. In these cases one is amazed at the ingenuity and activity with which the child, using what muscles he has, braces and turns and twists to escape painful positions. Indeed, in two instances where both legs, both arms, back, anterior, and posterior neck muscles were paralyzed, the hopeless attempt at defence was limited to wagging the head from side to side and feebly whining.

In sharp contradistinction to these types are the few individuals who with limited or extensive paralysis do not seem to be at all sick.

As a rule, unless definite ocular palsies are present, the eyes present no abnormalities. Sometimes photophobia is marked. The ear drums are normal in the great majority of cases. An occasional slight reddening along the handle of the malleus or about the margin of attachment has been seen. The throats, on the other hand, have usually shown a considerable degree of congestion. The tonsils have been, as a rule, enlarged but not reddened. In many cases they have appeared ragged and swollen. The tongue, in most instances, is covered with a delicate pale gray coating and sometimes heavily furred. Some of the younger children have been cutting teeth. Herpes labialis appeared only once or twice. The superficial lymph nodes were always palpable and often enlarged. It is, of course, not justifiable to lay too much stress upon glandular enlargement in children, but the finding has been very constant, and it is of interest that recent pathological studies have shown that a general adenopathy belongs to the disease.

Two interesting features of poliomyelitis associated with the neck have attracted our attention. As is generally described, rigidity of the posterior muscles has been almost a constant finding. It has not always been possible, however, to be sure whether this rigidity was of reflex origin or due to a calculated effort on the child's part to prevent anterior flexion. This question will be more fully discussed subsequently. The cervical rigidity has varied in degree from sufficient stiffness to permit lifting the whole trunk by the head to an almost normal flexibility. Most cases are markedly stiff and occasionally rigidity and retraction of the neck is as well developed as in a true meningitis (cases 2 and 3, pages 123 and 125). The other neck phenomenon has been a peculiar weakness of the anterior neck muscles, apparently not associated with a definite paralysis or even paresis. Very many of the children have shown it in the first few days of the disease. If the patient, lying in the dorsal position, is lifted by the shoulders, the head hangs back. Apparently no effort is made by the patient to raise the head so that it may follow the shoulders and trunk. Sometimes a slight effort may be made, but soon the head is allowed to drop back. This may depend on weakness of the muscles, but it is also possible that the

child gets some momentary sense of comfort, of which he takes full advantage, from the hyperextension of the neck which the manipulation permits. When the sign has persisted after the first or second week, we have usually thought that a definite paresis or paralysis was present.

Except in the cases where some part of the respiratory mechanism is disabled, the thorax has shown no abnormality caused by the disease. But the picture of the respiratory paralyses has been very striking. A description of them, however, does not belong here and will be given in the section covering the paralyses in general. The lung findings also have not been abnormal save in the cases with weakness of diaphragm or intercostals; but in those instances there have occasionally been found extensive areas of moist rales resembling in considerable detail the sounds of an acute pulmonary edema. These patients have had no variation in temperature at the time when the rales were most conspicuous. The significance of these sounds with a consideration of certain other vasomotor phenomena will also be discussed in the section on paralysis. Frank bronchopneumonia appeared in two of the thirteen cases with weakness of the respiratory muscles. In both of these the temperature continued high to the end.

The abdomen, like the thorax, is rarely the site of abnormality. There is often a slight distension, due perhaps to the same cause that underlies the constipation so commonly met with. The belly is soft and not tender. When the recti or lateral muscles are paralyzed there is sometimes a peculiarly lax, inelastic feeling to the abdominal wall. In these cases, also, a startling protrusion of part of the parietes may appear when the child cries. In the younger children, the liver is palpable, as becomes their age, but it is never tender nor pathologically enlarged. The spleen likewise shows no constant clinical abnormalities.

A curious thing is the unheralded advent of the paralysis. One may observe in the morning that a child moves its arms easily; a few hours later, on going to the bedside for some other purpose, the patient is found lying quietly as before, but when he rolls over, one arm falls back limp. The child seems unaware of the loss of power. In a few cases patients have complained of pain in an

extremity shortly before paralysis supervened. While the presence of paralysis is easily detected in older children, it is sometimes most difficult to find in infants. In any case, the most satisfactory method of beginning a search for muscular weakness is to sit down by the bed and watch the child for many minutes. Of course, in some instances, the patient lies a limp, inert form, the subtle expression of vitality gone. In such cases a glance is enough to determine the presence of paralysis, and more careful examination is needed only to learn its extent. If the pain or tenderness is not too intense, gentle handling of the extremities soon discloses the muscle groups that still have some power left in them. Older children will usually move the arms or legs upon suggestions calculated to demonstrate faulty motion. Often, however, when there is great pain, it is hard to tell whether the children will not or cannot move. In such cases the physician must decide whether the demonstration of the presence of a paralysis is of sufficient importance to justify his causing the patient the pain involved in such demonstration. In such cases the only way in which the presence of a paralysis may be accurately determined is by pricking the skin and determining whether or not the child draws the part away from the source of discomfort. For instance, if it be suspected that the deltoid is weak, the skin should be pricked on the posterior and inner aspect of the arm, when, if the deltoid is not involved, the arm will be drawn outward and upward. The extremity should be so placed at first that the suspected muscle will have to work against gravity; but it is surprising how quickly and skillfully the smallest infants turn and twist to make use of this natural force. Occasionally the presence of tone in a muscle can be shown by putting it suddenly on the stretch. For example, if the flexed forearm be sharply drawn down by the examiner toward extension, definite resistance will be noticed when the biceps is normal.

There is one group of cases in which it is almost impossible to locate definitely the paralysis. These patients present all the other features of the disease but no paralyzed muscles can be found. If such children be stood on their feet, however, they suddenly buckle at the hips and fall in a heap on the floor. Probably weakness of

the gluteal muscles in some cases is accountable for this form; in others, weakness of the quadriceps.

Müller states that paralysis of the intrinsic back muscles is of frequent occurrence. This is a difficult palsy to demonstrate in the acute stage of the disease; for in the great majority of instances, the children will not or cannot sit up at this time. In some cases pain makes the patient antagonize the effort to sit up; in others there is apparently such general weakness of trunk and neck that the head drops and the spine bends like a reed. Yet in a week or two these children may be sitting up straight in bed and playing actively.

The superficial and deep reflexes have shown considerable variation, but, on the whole, they have had a tendency to be present in the acute stage of the disease. This applies, of course, to the muscles where paralysis does not prevent response. The following table is made from a group of thirty-seven cases in which the power of response to patellar tendon reflex existed in one or both lower extremities.

TABLE OF KNEE JERKS.

Paralysis.	Number of cases.	Right knee jerk.		Left knee jerk.		Both knee jerks.	
		Present.	Absent.	Present.	Absent.	Present.	Absent.
Upper extremity and respiration alone	11					5	6
Bulbar	5					4	1
One lower extremity, either with or without upper	21	10		4		3	4
Total	37						

Knee jerk (one or both) present in 26 cases.
Knee jerks (both) absent in 11 cases.
Knee jerks (both) exaggerated in 3 preparalytic cases.
Knee jerk (right) present, knee jerk (left) absent in 1 preparalytic case.

It is evident that the knee jerk on the unaffected limb is more often present than absent. In eleven cases where paralysis was limited to the upper half of the body, the knee reflex was positive on both sides five times and negative six times. In eighteen cases of lower monoplegia, the knee jerk appeared in the strong leg fourteen times and was absent in four instances. Three cases with paralysis in muscles below the knee only had retained knee jerks. Of the four preparalytic cases, three presented bilateral exaggera-

tion; in one case the right reflex was present and the left absent. In a few cases the disappearance of a knee jerk, which had previously been active, has preceded paralysis of the extremity. However, absence of a knee jerk has not always meant loss of muscle power. Perhaps of more significance from a prognostic standpoint than the disappearing knee reflex at the beginning of the disease, is its reappearance in the period of improvement. Often the earliest sign of returning power is a slight jump of part of the quadriceps femoris when the patellar tendon is tapped. Occasional cases have shown greatly increased knee jerk on the strong side, when the reflex in the paralyzed leg is absent. This exaggeration has usually been more striking in the period of improvement, and sometimes has been associated with a crossed adductor reflex.

The appearance of a cutaneous eruption, which has been described as part of the disease, we have not seen. There have been several cases having more or less extensive erythematous rashes about the neck and chest, but these have looked like prickly heat and have had no constant distribution. Some of the children had been badly bitten by mosquitoes and many had pediculosis. Many cases have shown a pinkish purple mottling of the paralyzed extremities, but none the extreme degrees of cold purple skin with edema that have been described. In one instance, giant urticaria appeared, but there has been no example of zoster.

Sweating has not been a constant feature in the acute stage, but numerous cases showed localized areas which were continuously covered by perspiration. Thus one child's hands and feet sweat profusely, but the arms and legs were dry. Several of the patients had sweating heads; in one case it was sharply limited to the left half of the face. A few of the children sweat generally and fairly profusely for a week or more. There was no constant relationship between sweating areas and paralyzed muscles. Several tests for sweating by local hot air baths failed to give any satisfactory results.

A few cases have had retention of urine so that catheterization was necessary. This condition never persisted more than a few days. In some instances, true retention did not exist but there was difficulty in starting the stream. Hot applications over the dis-

tended bladder, or placing the child on a chamber containing hot water usually sufficed to induce micturition. In one case with stupor there was incontinence.

Constipation has been the rule. In only one patient was there any indication of loss of rectal control. This was in the case of an intelligent eight year old boy who, when constipated, had no difficulty; but when, as the result of catharsis, the stools were very loose, he had involuntary movements on several occasions and did not know it.

Pain in some form is a constant feature of the acute stage of poliomyelitis. In general, three types are found: spontaneous pain, pain caused by manipulation, and tenderness to pressure of the muscles and nerve trunks. These are not all equally common. Pain caused by passive motion is most frequent and seems to depend primarily upon anterior flexion of the spine. The clearest demonstration of this fact occurs when a child's trunk is bent ventrally, shoulders toward hips, to throw the spinous processes apart in preparation for lumbar puncture. Such a procedure brings about immediately a marked degree of anterior spinal flexion and is strenuously objected to by the patient. The entrance of the needle is often unnoticed. There are several other manipulations, like the test for stiff neck and Kernig's sign, which necessitate more or less bending of the spine anteriorly. The ingenious and active efforts of the children to thwart any motion which involves the least bending forward of the spine, or indeed diminishes a slight protective opisthotonos, have been very striking, so much so that we have been led to believe that the stiff neck of poliomyelitis differed from that of meningitis in being voluntary rather than reflex. With the Kernig's sign also, the voluntary element, where retained muscle power permits, is even more definite. If flaccid paralysis of a lower extremity makes resistance impossible, there is always complaint of pain when extension is carried until the buttocks begin to rotate forward and upward; but when the muscles have power to act, resistance to hyperextension is definitely voluntary and has not the feeling of reflex spasm. This painful bending of the spine is also often responsible for the unwillingness of children to begin sitting up in bed. The symptom occasionally persists for sev-

eral weeks and, in these instances, is the only thing that keeps a happy, healthy looking child flat in bed. It is interesting to watch these patients on their backs, playing cheerfully and actively with arms and hands, suddenly look glum, apprehensive, and suspicious when a move is made toward them that may mean raising their shoulders and heads from the pillow. Often early improvement in the paralysis is masked by this painful symptom, or the fear of it (cases 4 and 5, pages 127 and 129).

Spontaneous pain sometimes occurs in poliomyelitis. This is much less frequent than pain on passive motion. Usually it follows the course of the nerves like a true neuritis. It may be very severe. Young children cannot definitely recognize limitation of pain to the course of a nerve and they complain, therefore, of distress in the whole leg or foot. Such pain may perhaps be more often present than is generally supposed, for it frequently requires much urging and even sharp prodding to make a child move an extremity which seems to be paralyzed, when in reality the muscles have power but are painful. In some cases a child will cry out with pain which seems to come in stabs and paroxysms. The duration of pain of this sort varies like most of the symptoms of the disease. As a rule, it rarely lasts more than a week. In the case of one adult, the pain was so severe that morphine was necessary on several occasions. With young children we have used codeine. The third painful feature of acute poliomyelitis is the tenderness of muscles to pressure. Sometimes merely a touch suffices, but usually the muscles must be seized between thumb and fingers and a little pressure made to produce pain. There is little doubt that this tenderness is in the muscles and is not a hyperesthesia of the skin, for rubbing the skin without pressing on the underlying muscles, or even pinching, causes no painful sensation. A few cases also have definite tenderness over the nerve trunks like a neuritis (case 6, page 131). In one adult with complete flaccid paralysis of the right lower extremity, who had also great spontaneous pain, pressure over the anterior crural trunk caused intense suffering. Headache is more a symptom of onset than of the acute stage.

From the discussion of fever in the section on prodromata, it is evident that an elevation of temperature in poliomyelitis is not only

a constant feature, but also of short duration. The day of disease has been calculated from the date either of vomiting or onset of feverishness. The following table is based on the temperature findings of fifty-four cases in the acute stage of the disease.

TEMPERATURE TABLE NO. 1.

Day.	105° F.	104° F.	103.5° F.	103° F.	102.5° F.	102° F.	101.5° F.	101° F.	100.5° F.	100° F.	99.5° F.	99° F.	98.5° F.	98.10° F
1														
2			1		2				1					1
3		1			2		2							
4				1	2			2		1		1		1
5						2		3				5		1
6						1	1					3		2
7				1					2			2		3
8	1											2		1
9												1		3
10												1		1

TEMPERATURE TABLE NO. 2.

2d Day.		3d–6th Days (Inclusive).		7th–10th Days (Inclusive).	
Temperature.	Number of cases.	Temperature.	Number of cases	Temperature.	Number of cases.
98° F.	1	98° F.	4	97° F.	1
100 6° F.	1	99° F.	9	98° F.	8
102.6° F.	2	100° F.	1	99° F.	6
103.8° F.	1	101° F.	8	100° F.	2
		102° F.	7	103.3° F.	1
		103° F.	1	105° F.	1
		104° F.	1		

Total number of cases = 54.

It is obvious that after the fourth day there are only eleven cases with temperature over 99° F., whereas there are twenty-five cases with temperature of 99° F. or less. Before and including the fourth day, fifteen cases had temperatures above 99° F., and only three 99° F. or less. Consequently, since most of the patients have come under our observation after the acute stage has begun, the charts in the hospital have shown only the end of the temperature curve. Many of the cases have had continued low temperature, varying from just above normal to 99° F. or 99.5° F. for several weeks. Possibly there is a slight relationship, at least in point of time, between the onset of paralysis and the fall of temperature. This is not especially definite, nor does there seem to be any particular reason

why it should be. A somewhat remarkable feature of the temperature in many cases is the fact that its presence is shown only by the thermometer. The patients do not seem feverish. The typical end of the temperature curve is a sharp lysis, lasting usually twelve to twenty-four hours. Some cases have a much slower fall, and the temperature abates very gradually through two, three, or more days. In these cases it occasionally runs on for two or three weeks at just above normal. All the temperatures were taken by rectum, however, so that a curve in the smaller children continuing between 99°, 99.5°, or 100° F. is hardly to be considered an abnormal elevation.

II. Cases without Prodromata in Which Paralysis is the First Symptom.—A glance at text-figure 1 will show that in the seventy-two cases analyzed, only 4⅕ per cent. began with paralysis. In these instances the children have been brought to us on the first or second day of the disease. Usually they have the aspect of patients who have begun in the ordinary way, for in almost every case the child has been found in a drowsy, irritable state, and sometimes has vomited within twenty-four or forty-eight hours after the appearance of the muscular weakness. In one instance, the typical tenderness on spinal flexion developed after a day or two and persisted rather longer than usual. In general, therefore, these cases do not differ essentially from the more common type of the disease. They are a most significant group, however, from the therapeutic point of view. With them there has been no warning, and checking a possible advance of paralysis is the only hope (case 7, page 133).

III. Cases with Remission of Symptoms and Delayed Paralysis.—In the prodromal period described above, the occurrence of remission in the symptoms was discussed. A somewhat similar phenomenon sometimes appears in the acute stage of the disease, but very rarely. Thus a child will become less drowsy rather suddenly and, after seeming to be better for a day or two, again become irritable and sicker than before (case 8, page 134). In one case in which the spinal fluid had shown marked improvement, and the clinical symptoms were distinctly better, there was a sudden change late in the disease. The globulin content of the spinal fluid rose to double plus and at the same time the patient became highly nervous,

trembled, and had almost a spastic condition of the muscles of the arms. A Babinski reaction was also present. Subsequently the case improved again and left the hospital with only a slight residual paralysis.

IV. Cases with Deep Stupor.—An important clinical type of the disease, about which a good deal of confusion in nomenclature exists, still remains to be considered. The term "cerebral" or "encephalitic" was applied by Strümpell, Wickman, Müller, and others to describe those few rare cases which have had spastic paralyses, dependent upon upper motor neurone lesions. Recently, however, there has crept into the literature a confusing use of the words cerebral and encephalitic. They have been applied rather loosely to cases of poliomyelitis which have had marked disturbance of the sensorium. In view of the accompanying paralysis, these cases properly belong to the bulbospinal group. The only clinical evidence to indicate that cases of the type about to be described are poliomyelitis is the paralysis, which is usually of the flaccid or lower motor neurone variety. Ultimately it may be proper to consider that profound disturbances of the sensorium in this disease are due to lesions of the silent brain areas, but we have no anatomical evidence as yet for this assumption. Consequently, if an anatomical classification is accepted for the disease as a whole, these cases must be grouped primarily according to the anatomical lesion indicated by their paralyses. Usually this is bulbar, but it may also be spinal. Profound stupor, however, is such a striking feature that it must be recognized in any system of classification; but in relation to a primarily anatomical grouping, it can merely be placed as the sub-heading of a clinical variety. Therefore it is simplest to consider these as cases of bulbospinal poliomyelitis with profound stupor.

In its manner of onset, this clinical sub-group of the disease does not differ materially from the usual type. If there is any difference, it is that drowsiness preponderates in the early days and gradually deepens into stupor. In one case this process occupied nearly a week. The patients, of whom we had four, were brought to the hospital in varying degrees of stupor. They may lie in a sort of coma vigil, the head and eyes drawn to one side, and the

eyes wide open and expressionless. The face has a peculiar waxy mask-like immobility, although no seventh nerve paralysis exists. The head is drawn back a little, and from time to time an expression of annoyance, almost distress, crosses the features. Now and then a faint twitching or tremor passes over an extremity or the whole side. Yet, despite the coma-like condition, the patient can be rather easily roused by handling or prodding. There is almost immediate response and objection in the manner which has been so often seen in the other cases of this extraordinary disease, a displeased, irritated whine, and a vexed shrugging movement of the shoulder forward and upward, conveying quite distinctly the child's wish to be let alone (figure 15). Other cases of this kind may show a more stuporous condition with partly closed eyelids beneath which moves a slowly rolling eyeball. These individuals may or may not have retraction of the head, and lie prostrated and somnolent. They bear a strangely similar resemblance to patients with tuberculous meningitis. Like the coma vigil cases, however, they can also be rather easily roused by manipulation or prodding, and lapse as quickly again into stupor when undisturbed. Still other patients behave as though heavily drugged, and carry out sharp commands by slow, lazy, intensely apathetic motions.

An elevation of temperature is usually present in these cases. It seems to bear little relation, however, to the degree of stupor. Thus, one individual with a temperature of 99.6° F. was far more stuporous than another with a temperature of 104.2° F. Furthermore, the first case had a rise of temperature during the period of awakening from the stuporous condition.

As a rule, the stupor clears with considerable rapidity, after a duration of from three to six days. In one instance the patient after four days awoke as though from sleep, looked about in a bewildered fashion and then said she wanted to go home. The other cases regained normal mental condition more slowly, but the process occupied only a few hours.

To differentiate these cases from tuberculous meningitis is sometimes almost impossible. If paralysis exists when the case is first seen, one inclines more perhaps to the diagnosis of poliomyelitis, but in the preparalytic stage there is often nothing to give a differ-

ential clue. It is in these cases that the want of a specific reaction for poliomyelitis in the spinal fluid is most keenly felt, especially if the first search for tubercle bacilli is negative. The negative von Pirquet reaction, which occurred in our cases, is also a distinct help towards ruling out tuberculosis. All of our cases of stupor have had lesions in the cord at high levels. Three had palsies of the facial nerve, and one had upper extremity paralysis. This case and one of the bulbar cases also had slight, transient lower extremity involvement. One individual had lost only the power to look down and to converge. The extraordinary thing about the inability to converge was that both internal recti acted well in conjugate lateral deviation of the eyes.

From these various general clinical expressions of the acute stage of the disease, the patients recover more or less in the same way. As the acute symptoms pass, and the child begins again to take note of its surroundings and becomes cheerful, the paralyses, although previously observed, assume greater significance. Some cases show improvement more slowly than others. The children, as a rule, first become less drowsy and irritable. They will put out a hand into an offered palm or answer faintly in monosyllables. With some individuals, this change of interest comes toward the end of the first week; with others, not for two, three, or sometimes four weeks. This awakening, as it may be termed, is usually the beginning of improvement. From this time on, the convalescence is steady. Some cases seem entirely well in a day or two, while others gain in health and cheerfulness more slowly for a week or longer.

It is in this period that certain interesting psychic phenomena have been observed. Some children have been sullen and unwilling to play or be played with. In many cases fretfulness and irritability have persisted, and these children cry on the slightest provocation and sob for a long while afterwards. Still other individuals, fewer in number, present a peculiar emotional instability. Often such children begin to cry for no apparent reason and a moment later, if their attention is diverted by some trivial occurrence, begin as suddenly to laugh and giggle in a typically hysterical manner (case 9, page 137).

In general, however, the children rapidly regain normal psychic

poise, although in some cases there is a nervous apprehension which lasts for weeks. Furthermore, they soon lose the tired, wilted look and become rosy and fat. It is often surprising to see how quickly a very sick child looks well again, and sits up in bed smiling and happy. In such cases, only when the coverlet is drawn back and the helpless extremities appear, does one fully realize what a damaging blow the infection has dealt.

THE PARALYSES.

We have endeavored to show that with the advance in the knowledge of poliomyelitis, there has been a gradual shifting of the point of view from which the disease is approached. While not many years ago paralysis was regarded as the essential feature, the one important element of the picture, and the accompanying general symptoms were more or less completely disregarded, the tendency of present medical thought is towards the conception of poliomyelitis as an acute infectious disease which often leaves in its wake a variety of disabling end results.

Poliomyelitis, then, is not synonymous with paralysis, and the rôle played by this secondary symptom in the clinical history of the disease is becoming relatively less important. The new knowledge of abortive cases and of the probable infectivity of the prodromal stage teaches us that satisfactory control of poliomyelitis must, in the end, depend on the recognition of cases wholly apart from the paralysis. Where, formerly, paralysis was the one diagnostic criterion, its onset may now be only confirmatory evidence of a previously suspected diagnosis. If, however, paralysis is no longer considered to be the whole disease, it is unquestionably the greatest part of it. Now, and until the complex problems of diagnosis and therapeutics are finally solved, the feature of the disease which will form the center of the picture will be the paralysis, its prominence being dependent in large part on our imperfect methods.

The paralyses, while not considered of such primary diagnostic importance as they used to be, throw a good deal of light on the extent of the pathological process in the individual case; they offer valuable diagnostic information in atypical cases; and they so frequently dominate the clinical picture and constitute the whole of the

problem for the attending physician that they deserve a somewhat detailed study. The relation between the clinical signs and the pathological process in the nervous system is not an especially close one. The clinical signs depend, of course, on the pathological lesions, but they do not represent with any accuracy the extent of the lesions. Post-mortem examination shows a pathological involvement of the gray matter over an area which is usually much more extensive than that represented by the muscular paralysis, and an involvement of the white matter which has usually called forth no symptoms at all. That large areas of gray matter can be affected without producing clinical symptoms may depend on too slight involvement, or on the fact that most muscles derive nerve fibres from several levels of the cord, and their functional capacity is thus endowed with a large factor of safety. The absence of clinical symptoms depending on lesions in the white matter, even when these are extensive, is a necessary result of the coëxistence of lesions of the anterior horn cells, for in the presence of a widespread lower motor neurone disturbance, evidence of upper neurone involvement will usually be masked. Thus, even when the typical anterior horn lesions of poliomyelitis are associated with a diffuse inflammatory reaction through the white matter of the cord, the predominating part of the clinical picture will be the flaccid paralysis of lower neurone destruction.

One of the most characteristic of the general features of the paralyses in poliomyelitis is their peculiarly unsystematic distribution. Thus, in one case both legs are paralyzed, in another one leg and one arm, in a third case there is a peroneal associated with a deltoid or perhaps a facial paralysis, bizarre combinations with no apparent anatomical or physiological basis. There is, of course, a definite tendency for certain parts of the cord to be involved more frequently than others. Lesions in the lumbar enlargement are by far the most common, and next in frequency are lesions in the cervical enlargement with resultant leg and arm paralyses. The fact that certain parts of the cord are more apt to be affected than others probably depends largely on the blood supply. The primary reaction to the virus being a perivascular infiltration, the extent of the inflammation will vary more or less with the size and number

of the blood-vessels. That the lesion is most marked in the anterior horns of the gray matter is due to the fact that here, about the large motor nerve cells, the circulation is more abundant than in the posterior horns or in the white matter. Again, that extensive lesions are more frequent in the cervical and lumbar enlargements than at other levels of the cord is explained on an anatomical basis by the fact that the blood-vessels are largest and most numerous at the levels from which the great nerve plexuses arise. Other characteristics of the distribution of the paralysis, the affection of individual muscles, the involvement of various groups of muscles, as well as the sparing of others, depend on the pathological lesion being located in the cord itself and not in the peripheral nerves.

In other ways, too, the clinical picture and the pathological anatomy of poliomyelitis may be harmonized. Thus some paralyses are complete and permanent. In these there must have been an actual destruction of all or nearly all the nerve cells from which the affected muscles received nerve fibres. At other times one sees what is a weakness rather than a paralysis. This may be due either to an interference with nerve cell function by the pressure of exudate and edema without actual destruction of cells, or, it may be due to the destruction of a limited number of cells. As Bing[30] says, "Anterior root lesions, . . . unless very extensive, merely weaken and do not completely paralyze the muscle, owing to the fact that, as a rule, the muscle is innervated from several roots." Another class of paralyses is characterized by its transient nature. Either they last only a day or two, or they may persist for several weeks and eventually disappear. These are apparently the result, not of a destruction of nerve cells, but of a profound disturbance of function by toxic influences, or by the pressure of exudate, edema, and hemorrhage. When the disease ceases to progress, and the reparative stage with absorption of exudate begins, there is a relief of pressure, and the cells are enabled to resume their normal function.

The general manner of the onset of paralysis has already been considered. Some cases pass through a short period of muscular weakness corresponding to the beginning of the pathological

[30] Bing, Compendium of Regional Diagnosis in Affections of the Brain and Spinal Cord, translated by Arnold, New York, 1911.

changes, but the onset is usually sudden and swift, and the damage is quickly complete. The history is apt to state that paralysis came on over night, but occasionally one obtains more accurate evidence as to the possible rapidity of onset. One of our patients, an adult twenty years old, after several days of prodromata, went to sleep at half past twelve in the afternoon. Until then she had moved her legs normally. At two o'clock, one and one half hours later, she awoke and found her right leg completely paralyzed. There was no progression of symptoms. The whole damage was done at one stroke. Other instances are on record of even more rapid onset. Not infrequently, however, the first paralysis is followed at an interval of hours or days by further advances, and in one typical class of cases the process, as we shall see, sweeps like a wave over the whole spinal cord.

A discussion of the relative frequency with which different muscle groups are affected is of value only when a very large series of cases is analyzed. We, therefore, quote the following table from Wickman. It represents 868 cases seen by him in 1905.

1. One or both legs	353
2. One or both arms	75
3. Combination of arms and legs	152
4. Combination of legs and trunk muscles	85
5. Combination of arms and trunk muscles	10
6. Trunk muscles alone	9
7. Paralysis of "the whole body"	23
8. Ascending paralysis	32
9. Descending paralysis	13
10. Combination of spinal and cranial nerves	34
11. Cranial nerves alone	22
12. Localization of paralyses not given	60

In 43.69 per cent. of the cases, the paralysis was limited to the legs. One or both legs were affected in 85.64 per cent. of all the cases. A second table prepared by Lovett and Lucas[31] gives similar information (see page 58).

In the following more detailed consideration of the various clinical types of lower motor neurone paralysis, we shall take them up from the anatomical point of view: first those dependent on cord

[31] Lovett and Lucas, *Jour. Am. Med. Assn.*, 1908, li, 1677.

	Lovett and Lucas.	Duchenne, Seligmüller, Sinkler, Starr.
1. Both legs	130	84
2. Right leg	216	134
3. Left leg	239	133
4. Right arm	5	30
5. Left arm	5	23
6. Both arms alone	0	9
7. All four extremities	3	51
8. Arm and leg, same side	15	48
9. Arm and leg, opposite side	7	10
10. One arm, both legs	2	15
11. Abdomen with other paralysis	6	

lesions, next those with combined cord and bulbar lesions, and then those of purely bulbar origin. Finally, we shall describe the rapidly progressive cases as a class by themselves.

Spinal Paralyses.

Statistical studies show that in the great majority of cases of poliomyelitis, muscles of one or both legs are paralyzed, and that in nearly one half the cases the paralysis is limited to the legs. This corresponds to a pathological lesion in the lumbar enlargement of the spinal cord, and especially between the first lumbar and second sacral segments. Either one or both legs or any individual muscle may be affected. In the upper leg the quadriceps femoris is most often paralyzed, and in the lower leg the anterior group of muscles, the peroneals, the flexors of the foot, and the extensors of the toes, are most commonly involved. Extensive leg paralyses are of very frequent occurrence, but complete and permanent paralysis is not the rule. More often the flexors of the toes are spared, or if they are paralyzed immediately after the onset, they are usually the first muscles to show a return of function. On account of this relative immunity of the extensors of the foot and flexors of the toes, toe drop and contractures of the foot due to the unopposed pull of one group of muscles form a complication difficult to overcome. The paralyzed limbs are often cool to the touch and there may be a reddish purple mottling of the skin. The feet especially are apt to be cold, and they may be covered with a cold sweat. In the paralyses of leg muscles, one sometimes sees, perhaps more often than else-

where, a remarkably late restoration of function after such a long period has elapsed that one has begun to fear that the paralysis was permanent. In our experience this has been especially noticeable in the flexors and adductors of the thigh.

Sphincter paralyses, dependent on lower cord lesions, are probably rare. Many cases have bladder disturbances, usually retention of urine, but the facts that this is transient, that it appears during the febrile period, and may begin before the onset of other paralysis, make it seem probable that this symptom is more often analogous to what is seen in other acute febrile diseases, rather than a result of cord involvement. The reports by other observers of more severe and more persistent sphincter disturbances make it almost certain that paralyses of the vesical sphincters do exist. A few of our cases have had to be catheterized, one during a period of several days. Bowel disturbances suggesting paralysis of the sphincters are much more unusual. In one case of an intelligent boy, it seemed that such a condition might be the cause of an apparent inability to control the bowels.

Next in frequency to paralyses of the legs are paralyses of the arms, depending on lesions in the cervical enlargement of the cord from the fifth cervical to the first thoracic segment. In our series of seventy-one ward patients, thirty had involvement of the arms, but, as is usually the case, the great majority of them had a leg paralysis also. In only four instances the legs were not affected. Two of these showed an arm paralysis only; one had, in addition, a transient facial, and one a transient diaphragmatic paralysis. Except where arm lesions form a part of a very general paralysis, they are usually unilateral (case 10, page 138). We saw but one instance of both arms being affected in the absence of any leg disturbance (case 9, page 137). There is a tendency, greater even in the arms than in the legs, for the paralyses to be limited to muscle groups. The shoulder muscles and more especially the deltoids are most apt to be involved. It is a generally recognized fact that the proximal muscle groups of the limbs, especially the upper limbs, are more apt to be paralyzed than the distal muscles, and that after paralysis, recovery is quicker and more certain in the distal than in the proximal muscles. The shoulders are paralyzed more

often and their recovery is less certain. Elbow, hand, and finger muscles are less frequently affected and show a greater tendency to regain their power. Here, in analogy with the legs, one finds the flexors of the fingers less often involved and more completely recovering than the extensors. Complete and permanent flaccid paralysis of the whole limb is unusual. A recovery of the flexors of the fingers almost always occurs. Transient weakness or a temporary paralysis of one or both shoulders, occurring as a part of a more general process, is often followed by complete return of function, but if the paralysis persists and becomes well established, it appears to have an unusually bad prognosis. No muscle seems to atrophy as fast as the deltoid. One rather peculiar symptom complex was observed in association with an arm paralysis. The child had at first a complete flaccid paralysis of the left arm, but it gradually abated, with return of power in the triceps and extensors of the fingers, and to some extent in the flexors of the fingers. With this was noted failure of the left pupil to dilate, narrowing of the left eye slit, hemicranial sweating, the left side of the face being nearly dry, and hemicranial vasomotor phenomena. These symptoms depended apparently on a lesion of the ciliospinal ganglion which is situated in the cord at the level of the eighth cervical and first thoracic segments. The flexor muscles which showed such a delayed return of power derive their nerves principally from the seventh and eighth cervical, and first thoracic segments (case 15, page 147).

When the pathological process is fairly definitely limited to the cervical cord, and the legs are not paralyzed, one often observes a tendency to hold the legs stiffly, an exaggeration of the knee jerks and ankle reflexes, and a peculiar transient spastic ataxia of the legs. These symptoms certainly suggest an upper neurone disturbance and it seems probable that they are due to an involvement of the pyramidal tracts or of Clarke's columns, where these pass through the cervical region.

Paralysis of the Diaphragm.—In the upper part of the cervical cord, in the third, fourth, and fifth cervical segments, and in general above the area from which the brachial plexus arises, lie the cells whose axones form the phrenic nerve and innervate the dia-

phragm. Owing to the fact that the phrenic nerve has a large number of roots, or to some peculiarly fortunate circulatory condition, comparatively rarely is this area so severely affected that the action of the diaphragm is interfered with. Among fatal cases, however, this is not true, for practically all cases that die of poliomyelitis, without complication, die of respiratory failure, and respiratory failure means paralysis of the intercostal muscles and the diaphragm. It is noteworthy that among our fatal cases the diaphragm was usually the last to be affected, paralysis of the intercostals often preceding it by many hours. Owing to the vital importance of the diaphragm, its paralysis makes a serious prognosis, but recovery not infrequently takes place. We have seen two instances of paralysis of the diaphragm lasting for over a week and ending in complete return to normal. In one case it was associated with a paralysis of both arms, and in the other with an extensive paralysis of neck, arms, abdomen, and legs. In neither of these children was there any marked respiratory disturbance, but the picture was perfectly typical. Respiration is wholly thoracic, and sometimes the accessory muscles of the neck come into play. The abdominal wall moves with each respiration, but instead of its normal inspiratory protrusion, there is the typical inspiratory retraction depending on the lax diaphragm. Firm pressure on the sides of the thorax fails to induce any abdominal breathing and causes rapid, labored respiration. A condition which may be temporarily confounded with diaphragmatic paralysis is the type of breathing sometimes seen in crying or sobbing children; respiration is usually irregular, thoracic, and associated with an inspiratory retraction of the abdomen. This, of course, passes off as soon as the child becomes quiet again (case 13, page 143; see also case 9, page 137).

Paralysis of the Intercostal Muscles.—The thoracic portion of the spinal cord, probably owing to its less abundant blood supply, is also comparatively rarely attacked, except in the overwhelming infections terminating fatally. In such cases there is usually a progression upward from the lumbar region or downward from the cervical region, and the paralysis of the intercostal muscles which follows the invasion of the dorsal cord is one of the factors that cause respiratory failure and death. While the majority of cases with

paralysis of the intercostals end fatally, we have seen three instances of recovery. In two there was an associated paralysis of both arms and legs, and in one other of legs and abdominal muscles. The two former still showed an intercostal paralysis on discharge from the hospital, several weeks after the onset of the disease. In the latter there was return of function. In a fourth, seen in the dispensary, the associated leg paralysis cleared up almost entirely, but a complete intercostal paralysis persisted. Atrophy of the chest muscles, narrowing of the chest, and protrusion of the abdomen make a peculiarly characteristic late clinical picture (see figure 16). In some cases there has been an association of intercostal paralysis with edema of the lungs. The presence of coarse, moist rales in both lungs in the absence of fever and of cardiac weakness, and in the absence of evidence of a bronchopneumonia at autopsy have made us feel that the process may possibly be one of vasomotor origin. The diagnosis of paralysis of the intercostal muscles is less simple than that of the diaphragm, for in many children the normal type of respiration is almost wholly diaphragmatic and suggestive of weakness of the intercostals. In typical paralysis of the intercostal muscles, respiration is wholly abdominal, the thorax moves very slightly, its movement on inspiration being downward and backward instead of forward and upward. There is sometimes a sucking inwards of the intercostal spaces and, in children with flexible chest walls, of the lower part of the chest on inspiration (figures 16 and 17). Pressure over the abdomen does not induce a thoracic type of respiration, but it causes rapid, labored breathing, an effect not produced by compression of the chest. Both intercostal and diaphragmatic paralyses predispose to the development of bronchopneumonia (cases 14, 15, and 16, pages 145, 147, and 149).

Paralysis of the Abdominal Muscles.—The statements of various observers show a great divergence of opinion as to the frequency of paralysis of the abdominal muscles. From a study of our own cases, we have felt that an accurate estimation of its occurrence is extremely difficult, for the diagnosis in an acutely sick child of a transient weakness or paralysis of the abdominal wall is by no means easy. It is readily simulated by soft, atonic abdominal muscles. Apart from these transient paralyses, which undoubtedly

occur frequently, and from the involvements in rapidly fatal cases, there is an interesting class of abdominal paralyses which usually persist. These consist in either local areas of weakness or general paralyses of the abdominal muscles on one or both sides. The external and internal oblique muscles and the transversalis are usually involved together. The rectus abdominis is usually spared. Sometimes all the muscular sheet outside the rectus is weak, and there is a bulging of the sides of the belly, but more often the paralysis is localized, and there is a circumscribed protrusion of the wall resembling an abdominal hernia. These paralyses are brought out more definitely by coughing, by crying, or by attempting to raise the body in bed (figures 18 and 19). Paralysis of the abdominal muscles may be the only symptom that persists.

Paralyses of the Neck and Back Muscles.—These have been most common in our patients in severe cases with extensive paralyses. In most of them there has been involvement of both arms and legs, associated with that of the back and neck. In one case, however, the only paralysis ever noticed was a transient affection of the neck (case 17, page 151). Müller calls especial attention to the great frequency with which paresis of the trunk muscles occurs early in the disease. We have found the back muscles very difficult to test early in the acute stage, for the children are often either so sick or have so much pain that they will not try to support the spine. Moreover, it is hard to distinguish whether weakness of the back is caused by a spinal cord lesion, or is merely a general symptom. In paralysis of the neck muscles, the child cannot support its head, which falls helplessly forward, backward, or to one side. Paralysis of the back muscles is either unilateral or bilateral. In the former case the child may be able to sit up, but the spine bends with its convexity towards the weak side. If the lesion is bilateral, the patient cannot support the trunk and it falls backward, sideways, or forward, so that the body doubles up on top of the legs. Except in a few very extensive cases, paralyses of the neck and back muscles tend to disappear early.

Bulbospinal Paralyses.

It is probable that in most cases of poliomyelitis the pathological changes in the central nervous system are much more extensive than the clinical picture would lead one to suspect, and that scattered areas of exudate or hemorrhage are often present in the medulla and pons. In a smaller proportion of cases, but not infrequently, these lesions are large enough and so situated as to produce definite symptoms by involvement of the nuclei of the cranial nerves. Most commonly the cranial nerve affections form part of a general process in which the cord is simultaneously affected, but in a small proportion of cases the lesions of the cord are so slight as not to cause paralyses, and the clinical picture is that of an acute bulbar paralysis. Most instances of combined spinal and bulbar paralyses have extensive lesions involving legs, arms, and regions supplied from the bulb. Localized processes in the bulb and upper cord are much less frequent. Both where the cranial nerve affections occur alone, and where they are associated with spinal cord lesions, they are almost always unilateral. Bilateral bulbar involvements are extremely rare. Pathologically, as we have seen, the spinal and bulbar paralyses have the same basis. Clinically, too, though they may present quite different pictures, it is impossible to separate them, as one type merges gradually into the other. The greater number of cases form the intermediate type with both spinal and bulbar paralyses. In the Swedish epidemic of 1905, in which there were 685 cases, Wickman saw forty-two instances of combined spinal and bulbar paralysis. In the series of seventy-one cases which we have observed in the hospital there were, excluding the fatal cases, twelve of combined paralysis. The fact that our figures are proportionately nearly three times as large as Wickman's probably depends on the possibility of closer observation afforded in a hospital. Many of the cranial nerve paralyses were comparatively slight and transient, so that constant watching of the children was necessary for their detection. It was quite noticeable that the bulbar paralyses occurring in association with spinal paralyses were much less severe and less likely to be permanent than when they occurred alone. As has been the experience of other clinicians, we have found the

facial nerve most often affected. A facial paralysis occurred in eight of the twelve cases with both cord and bulb lesions, but in almost all it was slight and disappeared quickly. In three of the four cases of purely bulbar paralysis, facial paralysis was present and improved very slowly. Either the whole facial nerve or one branch may be affected (figures 20, 21, and 22). Ocular palsies were present four times in the hospital cases. In agreement with the findings of Wickman and Müller, we have found an abducens paralysis to be the commonest ocular palsy. Paralysis of the ocular muscles was sometimes transient, but often apparently permanent. One case seen in the dispensary had at first a paralysis of the right leg and of the right external rectus. Four weeks after the acute onset of the disease the leg had almost completely recovered, and the only evidence of the disease was a well marked internal squint. It is perhaps worth considering whether slight attacks of poliomyelitis may not be a more frequent cause of strabismus than is usually recognized. More extensive palsies and even complete ophthalmoplegia externa have been reported by Wickman. Nystagmus was noted several times in the early part of the acute stage of the disease. The eye grounds were examined in many of our cases, but we found no abnormalities of the optic nerve. While Wickman has reported one instance of optic neuritis in an acute case, and Tedeschi (quoted by Wickman[32]) found complete blindness and optic atrophy of the left eye in a chronic case, Müller failed to find any evidence of optic neuritis or choked disc in a large number of acute cases, and he believes that if either is found, the case is almost certainly not one of poliomyelitis. Disturbances of speech and phonation are quite frequently met with; the latter may be of all degrees from hoarseness or slight weakness to complete aphonia. They usually occur very early in the course of the disease and are transient, though we have seen them become progressively worse during the second week, and in one case the mother said that the child could not talk for nine days. Difficulties of deglutition are sometimes seen at the acute onset of the disease. This may be a transient symptom which lasts a day or two and consists only in an inability to swallow solids. In other patients, as

[32] Wickman, Die akute Poliomyelitis, *loc. cit.*

in two purely bulbar cases to be described, there may be total inability to swallow, lasting for many days and necessitating feeding by gavage. While the situation appears at the time to be most serious, the tendency to recovery, according to our experience, seems to be much better than one would expect, and return of function may take place even after ten days of paralysis. In progressive fatal cases, a paralysis of swallowing is frequently seen. Disturbances of speech and disturbances of swallowing often occur together. With paralysis of deglutition may be associated a paralysis of the hypoglossal nerve. This is usually a unilateral lesion causing inability to protrude the tongue straight, but cases of bilateral involvement with complete inability to protrude the tongue are reported. In the two of our cases which had prolonged difficulty in swallowing, but which finally became normal in this respect, a persistent hypoglossal paralysis remained as a residual lesion. In a dispensary case, the only bulbar symptom remaining several weeks after the onset was a deviation of the tongue with an atrophy of one half of it. Atrophy of the intrinsic muscles of the tongue occurs quite rapidly after hypoglossal paralysis, but no interference with function is apparent. Evidence of involvement of the vagus nerve in poliomyelitis will be shown in the consideration of the progressive fatal cases. Two fatal cases showed spinal fluids which had an exceptionally high power of reducing Fehling's solution. One of these had also a glycosuria. It is possible that an inflammatory lesion between the nuclei of the eighth and tenth nerves had the same effect as the "sugar puncture" of Claude Bernard and caused a hyperglycemia.

One of the most interesting clinical forms in which poliomyelitis appears is that of the purely bulbar paralysis. Until Medin's publication, the relation of cases with affections limited to the cranial nerves to the typical poliomyelitic cases had not been definitely brought out. There is now, however, abundant evidence, both pathological and epidemiological, to show that many cases formerly called acute bulbar paralysis are, in fact, poliomyelitis with a localized lesion in the pons and medulla. This type of case is not especially uncommon. We have had four instances under observation in the hospital and have seen other acute cases in the dispensary.

We have already called attention to the fact that the paralysis in these cases tends to be more severe and more lasting, than are the bulbar paralyses when they occur in association with general cord lesions. Except for the residual paralysis, however, the prognosis seems to be good. One might expect to see death from involvement of some of the higher centers, but this has not occurred in our experience, nor have we noted any tendency for a bulbar paralysis, once definitely established, to spread to the phrenic or upper cervical centers. Any of the cranial motor nerves may be affected, and the combinations in which various nerves are involved are numerous. As usual, the facial nerve is most frequently paralyzed, and a facial palsy may be the only evidence of the disease. In one case we found a paralysis of the sixth, seventh, and twelfth nerves, and complete inability to swallow. After being fed by gavage for five days, the patient regained the power of deglutition, and later the strabismus disappeared. The facial and hypoglossal paralyses persisted. In another bulbar case the only evidences of paralysis were inability to swallow, deviation of the tongue, and hoarseness of the voice. Feeding by gavage was continued for ten days. During this time the patient's general appearance became worse and his hoarseness was suddenly succeeded by almost complete aphonia. At this time, when he was very weak, sixteen days after the onset of the disease, he suddenly showed some return of the ability to swallow, and small amounts of food were given by mouth. Recovery of voice and deglutition proceeded uninterruptedly and the child was discharged perfectly well except for a residual paralysis of the right hypoglossus nerve. Quite characteristic of these high lesions has been an ataxia which has become evident when the children were well enough to begin to use their arms and legs. Knee jerks and Achilles reflexes are greatly exaggerated, and the legs may be held stiffly. The gait suggests somewhat a spastic ataxia. The children have difficulty in balancing themselves, a well marked Romberg sign may be present, and they walk with rather stiff legs and feet spread wide apart. This is a temporary condition and, after they have been on their feet for a few days, the gait becomes normal, but the exaggeration of reflexes often persists much longer. The cause of this phenomenon is difficult to determine with accuracy.

It may be due to lesions in the cerebellum, in Clarke's columns, or in the pyramidal tracts as they pass through the bulb or upper cord (cases 18, 19, 20 and 21, pages 152, 154, 155, and 158).

Rapidly Progressive Cases.

From the pathological point of view, there is no reason for considering separately those cases of poliomyelitis in which there is a progressive involvement of the nervous system, for the lesions are in themselves similar to those found in other cases. Clinically, however, they have formed such a characteristic group in our experience that it is convenient to discuss them by themselves. They constitute the greater number of fatal cases. Many, if not all cases showing the symptom-complex known as Landry's paralysis, belong to this group of poliomyelitis. There are in general two ways in which death occurs in poliomyelitis. Either it is due to a complication, for the most part bronchopneumonia, arising in the already injured system, or it is due to paralysis of the muscles of respiration. We have seen that a patient can live and can even recover after a paralysis of either the diaphragm or the intercostal muscles. When both become paralyzed, respiration ceases and death ensues. The fatal issue depends not on the nature of the pathological process, but on its location. If certain vital parts are spared, the extent of the disease seems to have little effect on the general health. The essential feature of death in poliomyelitis is that it is a respiratory and, in a way, a mechanical death. In most other acute infections we regard death as being due to a toxemia. The body becomes overwhelmed by a poison which interferes with its functions, until finally some organ, usually the heart, weakens and fails. That a toxic substance plays some rôle in the destruction of the nerve cells in poliomyelitis cannot at present be disproved, but that anemia and pressure play a very striking part is certain. The typical clinical picture, moreover, as we have seen it in the severe, fatal cases, is not that of a patient dulled by a general toxemia, but of one with a clear, alert sensorium, fighting for every breath until he is literally suffocated. This does not mean, of course, that there are not other cases of poliomyelitis which pass from somnolence to stupor and die without regaining consciousness. Whether, in such

cases, the mental condition is a toxic affect, or whether it, too, may not also depend in large measure on the mechanical action of the poliomyelitic process involving the centers of consciousness, is a question which we feel cannot be answered at present, but there is much to make the latter suggestion seem not improbable.

The average mortality for different epidemics of poliomyelitis varies between 10 and 20 per cent. Of the cases which we have followed in the hospital ten, or about 14 per cent., died. In three of these death was due to bronchopneumonia supervening on an extensive paralysis which included either the intercostal muscles or the diaphragm. In seven cases there was no evidence of any complication, and death was the result of poliomyelitis with paralysis of the respiratory muscles. We have seen that in most cases of poliomyelitis the paralysis is sudden and severe, and reaches its maximum in a comparatively short time. The same may occur in fatal cases. In one case seen outside the hospital, there was a sudden overwhelming paralysis which caused death within five hours after its onset. In the majority of cases, however, after the acute onset of a more or less widespread paralysis which usually involves either the intercostals or the diaphragm, there is an interval of a few days with a pause or a slower progression in the paralysis until there is a weakening and a final cessation of respiration.

The typical Landry's paralysis is an ascending paralysis involving first the legs, then the intercostals, arms, neck, and diaphragm. In most cases the picture is not quite so clear cut, for the primary paralysis is more extensive and involves both arms and legs, but in six of the seven uncomplicated, fatal cases, the intercostals were first affected, and death followed the failure of the diaphragm. In the three cases which had a terminal bronchopneumonia, however, there was a primary paralysis of the diaphragm. The majority of the uncomplicated cases conformed closely to the ascending type of Landry's paralysis. One only was of the descending type with early paralysis of the diaphragm and terminal involvement of the intercostals.

In the fatal cases, then, as well as in those that recover, there is usually a pause after the acute onset of the paralysis. There may be one or two days without any definite increase in paralysis, but it

is frequently noticeable that the children are not doing so well as those who will eventually recover. Often the respiration is more rapid and a trifle more difficult than the degree of paralysis warrants. They are frequently unusually nervous, excitable, and irritable. Then the paralysis may begin to increase. A laryngeal disturbance with hoarseness, aphonia, or difficulty in swallowing may be the first evidence of the spreading lesion. A weakening of the remaining muscles of respiration soon follows. If the intercostals are still active, the movement of the chest becomes less marked. If the diaphragm has hitherto been intact, its movement, as represented by the abdominal wall, becomes weaker, or there is an asymmetrical movement suggesting a paralysis of one side of the diaphragm. Respiration becomes more rapid, more labored, and perhaps irregular. Instead of the normal smooth, rhythmic cycle, there may be a sharp, jerky, forcible movement of the abdominal wall on expiration. Pressure over the abdomen causes distress and interference with breathing. The alæ nasi dilate with inspiration, and the accessory muscles of respiration in the neck begin to come into play. As the diaphragm weakens, the neck muscles become more and more prominent until it seems as if the whole work of breathing depended on them. The sternomastoids pull on the clavicle, and in one case they caused a partial dislocation of the sternoclavicular joint with each inspiratory effort. The head is thrown back, and with every breath the lower jaw is pushed forward and downward in a gasping attempt to get air. Occasionally, there is typical Cheyne-Stokes respiration, probably dependent on a lesion of the nucleus of the pneumogastric nerve. Meanwhile the lungs may have remained perfectly clear until the very end, or a few hours before death coarse, moist rales may accumulate, an edema suggesting vasomotor paralysis. The heart is sometimes strong and regular until it stops. Usually it continues beating for a considerable period after the cessation of respiration. Heart sounds have been audible for as much as five minutes after breathing stopped, and electrocardiographic records showed that stimulus formation continued for twenty-three minutes after cessation of the sounds. Several times a characteristic arrhythmia has set in for the last few hours of life. The heart beats perfectly

regularly and then suddenly changes its rate; jumps from perhaps one hundred per minute to one hundred and forty, remains regular at this rate for a few beats and then, possibly with a few interpolated irregular beats, drops back again to its former slow rate and regular rhythm (figure 14). In one instance the rate dropped from eighty-four to thirty-six and then varied between these two. This rhythmic arrhythmia may persist or it may give way to a regular rhythm and return later. It is apparently a so-called sinus-arrhythmia, and probably represents a vagus nerve disturbance. Records taken with the electrocardiograph show a condition which is quite similar to that described by Luciani as occurring in the heart of the asphyxiated frog. It is interesting that in one case, in association with the institution of artificial respiration and a lessening of cyanosis, the irregularity of heart action completely disappeared.

Fever was present in all of the fatal cases when they were admitted to the hospital. In most of them it varied between 101.5° and 103.5° F. In two cases it did not run over 100° F. The course of the temperature, however, was not unlike that seen in cases ending in recovery. In general, it was highest on the first day of paralysis, lower on the next day, falling approximately to normal by the day of death. There has been no tendency for the usual fever curve to be prolonged in the fatal cases.

The most remarkable feature of our fatal cases was the condition of the sensorium. Three of our patients were so young that observations on their mental state were not of value, but four, between the ages of three and a half and ten years, showed a very interesting and comparatively constant picture. We have already called attention to the apparent absence of toxic effects in many cases of poliomyelitis, and this is nowhere more strikingly illustrated than in these severe, fatal cases. During the prodromata, and often during the acute onset of the early paralysis, the children may be sleepy and drowsy in the manner which is characteristic of so many cases. This condition is, however, apt to be mild and transient and it is often soon replaced by a clear mental state. With the onset of respiratory difficulty, it seems almost as if the children were suddenly awakened and made to realize the struggle before them. Little children seem to age in a few hours. One sees a heedless,

careless, sleepy baby become all at once wide awake, high strung, alert to the matter in hand, and this is, breathing. The whole mind and body appear to be concentrated on respiration. Respiration becomes an active, voluntary process, and every breath represents hard work. The child gives the impression of one who has a fight on his hands, and who knows perfectly how to manage it. All he wants is to be left alone, not to be interfered with, to be allowed to carry out his fight on his own lines. Instinctively he husbands his strength, refuses food, and speaks, when speech is necessary, quietly and with few words. One little child of four, so helplessly paralyzed that she was unable to move, but with a mind that seemed to take in the whole situation, said to the nurse clearly but rather abruptly, between her hard-taken breaths, "My arm hurts"; "Turn me over"; "Scratch my nostril"; and then when the doctor approached, "Let me alone, doctor!"; "Don't touch my chest." Pressure on the chest, tight neck bands, anything that obstructs easy respiration is immediately resented. The child demands constant attention, is irritated unless everything is done exactly as he wishes it, and often shows an instinctive appreciation for some especially efficient nurse. He is nervous, fearful, and dreads being left alone. The mouth becomes filled with frothy saliva which the child is unable to swallow, so he collects it between his lips and waits for the nurse to wipe it away. He likes to have his lips wet with cold water, but rarely attempts to take it into his mouth, for he knows he cannot swallow it. During the whole course it is remarkable that cyanosis is absent. There is a little bluish tingeing of the lips and tongue, but much more distinctive is the pallor, which is sometimes striking. Sweating is profuse. Then, as respiration gets weaker, the mind becomes dull, and with the occasional return of a lucid interval, he gradually drifts into unconsciousness. An hour or more later respiration ceases. This peculiarly alert, keen mental state has been much less noticeable in small babies. They tend to be dull and drowsy most of the time; but in the older children this alertness has been such a characteristic feature of the fatal cases, that we almost preferred to have a child brought to us in a stuporous condition, rather than with a mind whose nervous acuity seemed due to a perception of impending danger (cases 22, 23, 24, 25, 26, 27, and 28, pages 160, 161, 163, 165, 168, 171, and 173).

Cases 29 and 30 (pages 174 and 176) died of a terminal bronchopneumonia.

Cerebral Type.

In a masterly article, Strümpell,[33] in 1885, first called attention to the analogy between certain forms of cerebral paralysis in children and poliomyelitis. For this group of cases he suggested the name polioencephalitis. In the twenty-four instances analyzed by him, the disease occurred in nineteen cases below the age of four. None of the children were over six years old. In general the onset was sudden, and there was an initial stage with fever, vomiting, and convulsions. The last was the most frequent initial symptom. A prodromal period seemed to be wholly wanting in certain cases; in others it lasted two or three days, and in some instances it extended over several weeks, during which there were constant convulsive seizures. After the prodromal period, the child was found to have a hemiplegia. The paralysis usually began to improve shortly, but a limp and, more often, disturbed function of the arm usually persisted. The face was less often and less severely affected than the arm or leg. Strabismus occurred in several cases. Monoplegic paralysis of arm or leg, or even an ataxia without actual paralysis, was occasionally the only disturbance. There was no atrophy of the muscles or reaction of degeneration. The reflexes were usually exaggerated. Athetosis, epilepsy, disturbances of speech and intelligence sometimes occurred as sequelæ. In his discussion of the analogy between this type of case and poliomyelitis, Strümpell says it is noteworthy that, "in both diseases the chief seat of the lesion is the gray matter, in one case in the gray matter of the anterior horns, and in the other in the corresponding portion of the cerebrum, the cortex."

When, in 1898, Medin[34] published the first comprehensive description of the epidemic form of poliomyelitis, he was able to include the reports of three cases with fever, somnolence, convulsions, spastic hemiplegia, and exaggerated reflexes, and of a fourth in which apparently both cerebral hemispheres were affected. In

[33] Strümpell, *loc. cit.*

[34] Medin, *Arch. méd. des enfants*, 1898, i, 257, 321.

two instances the sixth cranial nerve was also paralyzed, and this bulbar affection formed a connecting link between the spinal and encephalitic forms. This, and the occurrence of both spinal and cerebral paralyses in the same epidemic convinced him that they had a common cause.

Harbitz and Scheel described a case of acute encephalitis in a man thirty-nine years old. After an onset with headache, fever, and sweating, stiffness of the neck, vomiting, and convulsions set in. The patient was unconscious. All the limbs could be moved, but they were rigid, and the patellar reflexes were exaggerated. The tongue deviated to the left. The man died, and at autopsy macroscopic circumscribed encephalitic or meningoencephalitic lesions were found in the right temporal lobe and in the gyrus fornicatus on both sides. Microscopically the lesion was more widespread and involved the central ganglia and medulla. In the spinal cord there was meningeal infiltration, but there was no definite inflammation of the anterior horns except in the cervical region. "Histologically, the inflammatory process resembled acute poliomyelitis in all its details."

These three sets of observations, the first two of which at least are of historical importance, give a fairly definite outline of what is to be considered as the cerebral form of poliomyelitis. The classification of this type rests, as far as possible, on an anatomical basis. These are cases in which the predominating pathological lesion is in the brain itself, and in which the clinical manifestations suggest a disturbance of the upper motor neurone. The identity of the cerebral form of the disease has, as we have already stated, been greatly obscured by the tendency of many authors, especially English and American, to use inaccurately the terms cerebral or encephalitic. The frequent application of these terms to cases which show certain general symptoms, such as stupor, delirium, or meningism, has led to much confusion. That the term cerebral as applied to this disease has reference to the supposed anatomical seat of the lesion, and that the cases form a fairly definite clinical group, is shown by a glance at the historical basis on which the classification has developed. It will often, however, be difficult to draw a hard and fast line between what shall be called spinal and what

cerebral cases. Clinically as well as pathologically, the types must merge. It would seem best to be governed by the most prominent symptoms. A case with a spastic hemiplegia would be classed as cerebral, even if it were associated with a slight nuclear paralysis of the sixth nerve; and a case with flaccid facial and arm paralysis would certainly be of the bulbospinal type in spite of showing a somewhat ataxic gait and exaggerated knee jerks, which might point to upper neurone lesions. After all, classification is purely artificial, and the real reason for making a separate class of these cases is to call attention to the fact that the virus of poliomyelitis may cause symptoms of cerebral rather than of spinal origin.

In spite of the acknowledged fact that small scattered lesions are not uncommonly encountered as autopsy findings in the brain in poliomyelitis, the occurrence of extensive cerebral involvement has rarely been demonstrated. Moreover, the final proof that typical cerebral paralyses may have the same etiology as poliomyelitis can be obtained only experimentally. As yet the virus has not been shown to exist in typical polioencephalitis by transference of the disease to monkeys. Anderson and Frost,[35] however, report that the blood serum of a patient who had a paraplegia of the legs, at first flaccid, but in a few days becoming spastic, was able to neutralize active virus. It is rather remarkable, too, that, in spite of the fact that monkeys are usually inoculated intracerebrally, the paralyses are always spinal and not cerebral. The evidence, however, of the identity of the two diseases is still largely clinical and consists in the finding of cerebral cases during the course of typical epidemics, and of finding both flaccid and spastic paralyses in the same patient. Thus both Möbius[36] and Hoffmann[37] saw instances of flaccid and spastic paralysis occurring in members of the same family. Much more striking, however, than that a number of such suggestive instances have been observed, is the fact that, with the tremendous increase in the incidence of poliomyelitis during the past few years, and with its more general recognition, there has not been a corresponding increase in the number of true cerebral cases reported.

[35] Anderson and Frost, *Jour. Am. Med. Assn.*, 1911, lvi, 663.
[36] Möbius, *Schmidt's Jahrb. d. ges. Med.*, 1884, cciv, 135.
[37] Hoffmann, *München. med. Wchnschr.*, 1904, li, 225.

The majority of those reported as cerebral or encephalitic cases do not conform with the anatomical standard that has been laid down. Wickman, who has probably had a wider experience than anyone, states that during the great Swedish epidemic of 1905 he did not see any definite cerebral cases. Krause, in the Westphalian epidemic of 1909, saw one case with encephalitic features. Zappert, whose studies include 555 cases, saw only five instances of cerebral hemiplegia. Müller reports four cases with spastic paralyses, but states that in none of them was there conclusive evidence that the condition was due to a polioencephalitis of the cerebrum. He believes that the symptoms depend rather on an involvement of the pyramidal tracts in the bulb or cord. One case only might have been due to a disseminated encephalitis,—a boy who developed a complete right-sided hemiplegia, and, in addition, a few days later, a left-sided spastic paralysis. Such a case would fall in line with the fact that the lesions of the brain as observed at autopsy are always bilateral.

In our own series of cases we have seen no example of a typical polioencephalitis. One case, however, showed a picture which was so suggestive of cerebral involvement that we are inclined to believe that it belongs to this group. A boy of four years and three quarters was taken sick with fever and vomiting. He became somnolent and when first seen was in an almost stuporous condition. The eyes showed a slight external strabismus on the left, and there was an inability to converge or to look downwards, which, according to our ophthalmological consultant, Dr. Schirmer, depended not on a lesion of the eye muscle nuclei, but on a higher coördinating center. There were no other paralyses. The Oppenheim sign was positive on both sides, and the Babinski reaction was suggestive. As the child came out of his stupor, the knee jerks became exaggerated, and a marked ataxia of arms and legs was observed. The diagnosis in this case was made more certain by the fact that the child's infant cousin, who had been in bed with him during his sickness, later developed a typical poliomyelitis (case 31, page 177).

During the past season, we have seen but one case of cerebral hemiplegia in a child. The conformity of the history and of the course of the process in this instance to the cases cited by Strümpell

were quite striking, but the chief interest in the case lies in the fact that, while occurring at a time when there was much poliomyelitis in the vicinity, we have good evidence in support of a different etiology. The child, a little girl of three years, hitherto perfectly well, was noticed to be feverish and out of sorts on the morning of October 4th. She played during the day but in the evening she had high fever, reaching 105.2° F., and after taking some milk, vomited. Shortly afterwards she was taken with convulsions which were so prolonged and so severe that the attending physician gave her some chloroform. When she quieted down, her left arm was found to be limp. The next day a left-sided facial paralysis, with paralysis of the left arm and leg, was observed. Improvement began quickly, especially in the leg. When seen by us, thirteen days after the onset, her left leg was practically well, but a well marked facial paralysis persisted, and there was ataxia and incoördination of the left arm. The knee jerks and Achilles tendon reflexes were active on both sides; there was no Babinski reflex and no Oppenheim sign. The eye grounds were negative. Lumbar puncture was done and the spinal fluid found to be absolutely normal. After a little over three weeks, a specimen of blood was taken, the serum mixed with active virus of poliomyelitis (0.1 of a cubic centimeter and 0.3 of a cubic centimeter in two experiments), the mixture incubated and injected into two monkeys. Both monkeys became sick at about the same time as the controls, and at autopsy showed lesions characteristic of poliomyelitis. The serum had no power of neutralizing the virus, and the case was, therefore, probably not one of poliomyelitis. The many features which this case has in common with those described as polioencephalitis, and its occurrence in association with other typical cases of poliomyelitis, made the test of considerable interest.

ABORTIVE TYPE.

The term "abortive" was applied by Wickman to those instances of poliomyelitis which do not develop paralysis. Certain border line cases with slight weakness but no true paralysis, Müller has called "*lavierte*" or "embryo" types. This seems perhaps an unnecessary distinction, especially in view of the new conception of the disease as a general systemic infection. As long as the cerebro-

spinal tract was held to be the only seat of the malady, and paralysis was considered its usual manifestation, the word "abortive" for unparalyzed cases was entirely satisfactory; but since recent developments in the pathology of poliomyelitis have demonstrated a general disease affecting the lymphatic apparatus and many of the parenchymatous organs as well, there is some chance of a misunderstanding of the term. The case which fortunately escapes paralysis is as much a true example of acute poliomyelitis as the paralytic, and as a source of contagion it is more dangerous. Indeed there are certain scattered facts gleaned during the past decade by clinicians and laboratory workers which, when brought together to bear upon the question, indicate that the non-paralyzed cases are possibly the more usual, and that the paralyzed ones are less frequent forms of the disease. Wickman, for example, found in his large epidemiological studies that the abortive cases represented from 25 to 56 per cent. of the total incidence of the disease, but he states that these figures are probably too low. Müller also believes such figures to be too low. He considers that the unparalyzed cases considerably outnumber the paralyzed. This view receives striking support from the recent demonstration of typical visceral lesions indicating that there is a general systemic infection. With such pathological findings, symptoms such as fever and malaise are reasonably to be expected. It may be that the abortive cases are those in which this general process is present, but in which the nervous system has been spared. Netter and Levaditi[38] have demonstrated that the serum of abortive cases neutralizes the virus *in vitro,* just as does the serum of the patients that develop paralysis. It is quite probable that in the past many of the abortive cases have been unrecognized and in certain cases at least, the apparent immunity of adults may be dependent upon such a previous, unrecognized attack. That the neutralizing substance in the blood may persist for a long period following an attack of the disease, and probably immunity be present as well, is shown by the case of a man who had been paralyzed thirty years before and whose serum still protected a monkey from the virus.

Since there is no essential difference, except in degree, between the

[38] Netter and Levaditi, *Compt. rend. Soc. de biol.,* 1910, lxviii, 617.

severe and abortive cases, a description of the symptomatology of the abortive type as a clinically separate group seems unnecessary. Wickman has attempted to classify the various forms of the abortive type, and groups them under four headings, thus:

1. Cases which run the course of a general infection.
2. Cases in which meningeal irritation is especially marked (meningism-like).
3. Cases in which pain is very marked (influenza-like).
4. Cases with gastro-intestinal disturbance.

Such a classification, based purely on symptoms, is perhaps as good as any other, but it is a grouping into which all cases of the disease can well be put during the time when diagnosis is especially difficult and really important. Actually there is little difference between the symptoms of the abortive cases and the prodromal symptoms of cases which become paralyzed, so that in solving the problem of diagnosis during the preparalytic stage, the possibility of recognition of the abortive case will be coincidently accomplished.

Our experience with cases which did not develop paralysis has been small numerically. Certain significant points, however, concerning the unparalyzed group have come to our notice. There seems to be in many cases some degree of muscular weakness. This may be transient. Two of our cases showed some indefinite weakness about the pelvis which made it difficult for the children to stand. They appeared to buckle at the hips and fall, following a sudden change of position or balance (case 32, page 180). Such cases belong perhaps more properly to Müller's group of "embryo" forms and serve, therefore, to emphasize the number of grades of severity that shade almost imperceptibly one into the other. The record of an abortive case (case 33, page 181) is given completely because the patient was under observation continuously from within twenty-four hours of the first symptom throughout the whole course of the disease. History of exposure, clinical picture, blood and spinal fluid examinations, and finally neutralization of virus M.A., all supported the diagnosis of poliomyelitis without paralysis. In this instance diagnosis of acute poliomyelitis without paralysis was fairly justified. Besides the history of exposure, the spinal fluid showed changes that were most suggestive. Furthermore, the clin-

ical picture was very striking, apathy and drowsiness being most obvious. However, it is difficult to select any one feature of the case as pathognomonic; a history of exposure is in itself extremely unreliable evidence. The following example is illustrative of this fact. Donald P., aged three years, suffered from malaise, anorexia, and drowsiness a day or two before his brother fell sick with a fatal attack of poliomyelitis, but his blood serum failed to neutralize the virus. While this is perhaps not absolute proof, still it should make one a little less ready to diagnose abortive poliomyelitis in every child who is a little indisposed in the summer. Two other examples of the abortive type that came to our notice were interesting because of the very slight muscular weakness that each displayed. If it had not been for the history of exposure, it is quite possible that both of these cases would have been overlooked. The weakness was so slight and consequently so difficult to locate, and above all so transient, that it very nearly escaped observation.

Of the other clinical features, it may be said that irritability and drowsiness are frequently present. The knee jerks are most variable. Wickman reports instances of disappearance of the reflex on one or both sides, and also cases where the reflex is exaggerated. Pain is a very common symptom, just as it is in the paralyzed cases. The pain, as described in the section on the acute stage, may be muscular or neuritic, often in the neck or back, or in the form of headache. The disease which, perhaps, the abortive cases most frequently resemble is influenza. Consequently, such cases occurring during the summer months, especially in the neighborhood of a patient with paralysis, should be viewed with suspicion and quarantined.

THE BLOOD.

The complete symptomatology of filterable virus diseases in man is not yet established. Certain clinical features of the two examples most studied, hydrophobia and poliomyelitis, have been well described, but the blood picture, which has been so thoroughly determined in most infectious diseases and is of such value in differential diagnosis, is little discussed. Owing to the resemblance of numerous features of rabies and poliomyelitis, it seemed that a comparative study of the blood changes in the two diseases might throw

some further light on the nature of the human body's reaction to invasion by infecting agents of this type. Unfortunately, however, despite the great amount of information that has been recorded about each of these diseases, only a few meagre and often conflicting statements are to be found on the subject of blood. The Systems of Medicine make no mention of blood counts in either malady. In one exhaustive report of thirty cases of rabies by Bain and Maloney[39] the blood of one case is reported to have had a "slight polymorphonuclear leukocytosis."

Through the courtesy of the attending physicians at the Presbyterian Hospital, New York, the following blood counts from cases of hydrophobia are available:

Patient I. Male, age 35 years. Temperature 99.3° F.

		Per cent.		Per cent.
	Polymorphonuclears ...	76.0	Transitionals	3.8
Leukocyte count 9,400.	Lymphocytes	10.5	Basophiles	0.0
	Large mononuclears...	9.8	Eosinophiles	0.0

Patient II. Male, age 27 years. Temperature 100–103° F.

		Per cent.		Per cent.
	Polymorphonuclears ...	76.5	Transitionals	2.5
Leukocyte count 13,000.	Lymphocytes	11.0	Basophiles	0.0
	Large mononuclears...	10.0	Eosinophiles	0.0

Patient III. Male, age 27 years. Temperature 103° F. (Nov. 26.)

		Per cent.		Per cent.
	Polymorphonuclears ..	85.0	Transitionals	3.0
Leukocyte count 34,450.	Lymphocytes	12.0	Basophiles	0.0
	Large mononuclears...	0.0	Eosinophiles	0.0

Patient III. Temperature 103° F. (Nov. 27.)

		Per cent.		Per cent.
	Polymorphonuclears ..	87.0	Transitionals	2.0
Leukocyte count 30,200.	Lymphocytes	10.5	Basophiles	0.0
	Large mononuclears...	0.5	Eosinophiles	0.0

[39] Bain and Maloney, *Lancet*, 1909, ii, 772.

Obviously, except in the first case where the total count was normal, there was a leukocytosis. In all the cases a definite polymorphonucleosis existed with a low lymphocyte percentage.

In the literature of poliomyelitis there are conflicting statements about the leukocyte count. La Fetra,[40] in New York, and Müller, in Germany, have made the most extensive observations on the blood. The former reported in six cases a leukocytosis, running from 13,400 to 20,600. He says nothing about the differential counts. Müller, on the other hand, reporting fifteen cases, says that a leukopenia of from 3,000 to 5,000 was always present in the acute stage. He gives no figures but states that there was a slight increase of the lymphocytes. Gay and Lucas made a comparative study of the blood in monkeys suffering from the experimentally produced disease, and of children with poliomyelitis. In monkeys they report a leukopenia during the acute stage with a lymphocytosis ranging from 10 to 20 per cent. above normal. The protocols of the human cases, however, do not support very convincingly their statements that a similar blood picture occurs in the human cases. The lowest count, that reported from a nine months' old baby, (case II) is 7,800 to 12,200. The other three cases showed the following leukocyte counts:

Case I, 20 months, 11,600–17,400.
Case III, 2 years, 12,000.
Case IV, 5 years, 13,400.

These can hardly be considered leukopenias. In two of their four cases, a lymphocytosis, "slight" for one, and "62 per cent." for the other, is given. Of the other two cases, one, a child of five years, had a normal differential count, and the other a polymorphonuclear cytosis of 75.5 per cent.

The following tables and the ensuing statements are based upon the study of the blood of our seventy-one hospital patients. The actual figures given represent the counts from only fifty-nine cases. The records of the other twelve cases were omitted because those patients suffered from other conditions which might have affected the blood count. Care was taken to avoid collecting the blood at

[40] La Fetra, *loc. cit.*

times when a digestive leukocytosis might have been present. The total white count was made in the usual way, the blood and 0.5 per cent. acetic acid solution being mixed in a 1 :10 pipette and the drop counted on a 0.1 of a millimeter cell. For differential counts, Wright's modification of the Romanowski stain was used.

The first series of tables has been arranged simply to record the counts by weeks of the disease and age of patients. The second series is designed to show minimal, maximal, and average counts for groups of cases. The composite table (page 95) shows minimal, maximal, and average total leukocyte counts of all ages together by weeks. Such a compilation seems justifiable, because the age variation is much less in respect to the total than the differential leukocyte count.

SERIES I.

Age 6 to 12 Months.

First week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.†
1	$\frac{3}{2}$* 25,300	59.0	23.5	2.5	13.5	0.5	0.0	1.0
2	$\frac{2}{0}$ 35,600	32.0	55.5	1.0	9.0	0.0	1.0	1.5
3	$\frac{3}{1}$ 23,900	25.0	57.0	2.0	15.0	0.0	1.0	1.5
4	$\frac{2}{5}$ 23,800	65.0	16.5	18.0	0.5	0.0	0.0	0.0

Age 1 to 2 Years.

First Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{7}{6}$ 15,300	36.0	50.0	2.0	7.5	0.0	5.5	0.0
2	$\frac{7}{5}$ 16,300	26.5	42.0	7.5	13.5	1.0	9.5	0.0
3	$\frac{1}{0}$ 15,000	52.5	31.5	13.5	1.5	0.5	0.5	0.0
4	$\frac{5}{2}$ 15,200	44.0	33.5	12.5	6.0	0.0	3.0	1.0
8	$\frac{6}{4}$ 15,700	53.5	28.5	12.5	4.5	1.0	0.0	0.0
9	$\frac{7}{5}$ 26,000	27.0	39.0	10.0	8.0	0.0	15.5	0.5
10	$\frac{6}{6}$ 18,200	51.5	29.0	2.0	9.0	0.5	8.0	0.0
12	$\frac{5}{3}$ 23,300	70.0	16.5	3.0	9.0	0.5	0.5	1.0
14	$\frac{8}{7}$ 17,500	42.5	47.0	1.5	5.0	0.0	3.5	0.5
17	$\frac{9}{5}$ 12,200	47.0	40.0	10.0	2.5	0.0	0.5	0.0
18	$\frac{5}{1}$ 17,100	38.0	49.0	1.5	11.0	0.0	0.0	0.5
20	$\frac{8}{6}$ 13,400	67.5	28.0	7.0	5.0	0.0	2.0	0.5

* The numerator of the fraction signifies the day of disease, the denominator the day of paralysis.

† "Stimulation forms" of Türck.

SERIES I (CONTINUED).

Age 1 to 2 Years.

Second Week.

Number of case.	Total leukocyte count.	Polymorphonuclears	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms
5	14,800	44.0	56.0	——	——	—	—	—
6	14,200	43.5	42.0	7.5	6.5	0.0	0.5	0.0
8	22,000	46.0	38.5	0.5	11.0	0.5	3.5	0.0
10	30,400	73.5	9.0	8.5	7.0	6.0	2.0	0.0
11	12,300	37.0	56.0	1.0	4.0	0.5	1.0	0.5
13	16,000	45.5	21.5	23.0	6.0	2.5	1.0	0.0
14	16,100	47.0	38.0	1.0	7.0	1.0	5.5	0.5
15	16,200	51.0	30.5	9.0	5.0	4.5	0.0	0.0
17	17,400	64.0	23.0	0.5	8.0	2.0	2.0	0.5
19	20,000	45.0	40.5	0.0	9.5	1.0	3.5	0.5
20	16,200	53.5	31.5	4.5	10.0	0.0	0.0	0.5

Third Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms
1	14,600	47.0	43.0	4.0	2.0	0.0	3.5	0.5
2	17,600	41.0	38.5	3.0	4.0	0.0	10.5	2.5
4	14,800	43.0	39.5	1.5	13.5	1.0	1.5	0.0
5	8,300	61.5	30.5	5.0	0.5	0.0	4.0	0.5
6	19,100	50.0	40.5	2.0	6.5	0.5	0.5	0.0
7	12,200	67.0	22.0	7.5	2.5	0.5	0.0	0.5
8	15,900	50.0	35.0	4.0	8.5	0.5	1.5	0.5
9	26,100	30.5	58.5	3.0	5.5	0.0	2.5	0.0
13		38.4	55.2	5.6	0.4	0.0	0.0	0.4
15	22,500	64.5	29.5	3.0	1.5	1.5	2.0	0.0
16	13,400	42.0	43.5	1.0	10.0	1.0	2.5	1.0
18	17,200	49.5	39.0	6.5	3.5	0.0	1.0	0.5
19	25,600	59.0	33.5	0.0	7.5	0.0	0.0	0.0

Fourth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	13,500	33.0	44.0	9.5	9.0	0.0	4.0	0.5
4	20,100	33.0	52.5	3.5	7.5	0.0	2.0	1.5
5	17,800	60.0	34.5	3.0	1.5	0.0	1.0	0.0
6	24,700	55.5	38.0	5.5	2.0	0.0	1.5	0.0
7	10,100	41.0	50.0	6.0	2.0	0.5	0.0	0.5
11	15,800	42.0	37.0	4.0	17.5	1.0	1.0	2.5
13	16,000	29.0	63.5	4.5	1.5	0.0	1.5	0.0
14	18,400	34.0	52.0	7.0	5.5	0.0	1.5	0.0
15	21,500	46.5	28.0	22.0	3.5	0.0	0.0	0.0
16	14,200	26.5	23.0	44.0	3.5	0.0	2.5	0.0
19	15,200	51.0	21.5	20.5	3.5	1.0	2.5	0.0

SERIES I (CONTINUED).

Age 1 to 2 Years.

Fifth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	20,300	60.5	20.5	10.0	2.0	0.5	6.0	0.5
2	23,600	42.5	26.0	13.5	10.0	0.0	6.5	1.0
4	9,100	40.0	42.0	7.5	7.5	0.0	2.5	0.5
6	21,600	38.0	56.0	1.0	3.5	0.0	2.0	0.0
7	13,100	38.5	43.7	8.0	8.5	0.0	2.0	0.0
8	16,400	43.0	42.0	8.0	3.5	0.0	2.5	0.5

Sixth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	12,600	37.5	49.0	0.0	9.5	0.5	2.5	1.0
7	9,600	40.0	32.0	10.0	15.0	0.5	0.5	2.0
11	26,700	23.0	69.0	6.0	0.5	0.0	1.5	0.0

Seventh Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
8	26,700	51.0	34.5	10.0	4.0	0.0	0.5	0.0
11	23,700	31.5	45.0	9.0	5.0	2.0	6.5	0.5

Age 2 to 3 Years.

First Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	15,300	56.0	30.5	8.5	3.0	1.5	0.5	0.0
2	23,000	56.0	20.0	10.5	12.0	0.5	0.5	0.5
3	18,400	32.5	43.0	14.0	9.5	0.0	0.5	0.0
11	13,800	47.5	31.0	1.5	7.0	0.5	7.0	1.0
12	9,800	64.5	21.5	7.0	3.5	1.0	1.0	1.5
14	14,200	58.5	31.5	7.0	3.0	0.0	0.0	0.0
15	10,600	49.0	28.0	2.0	18.0	0.5	2.0	0.5

Second Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
5	16,400	38.0	41.0	5.0	10.5	0.5	4.5	0.5
6	15,900	60.0	10.0	23.5	5.5	0.0	1.0	0.0
7	9,800	61.5	21.5	2.0	7.0	0.0	7.5	0.5
9	20,000	57.0	23.5	8.5	4.5	0.0	6.5	0.0
10	12,400	54.0	28.0	7.0	5.0	0.0	5.0	0.0
11	15,200	47.6	31.3	14.0	2.0	0.0	0.5	0.0
13	16,600	53.0	34.0	4.0	3.5	5.0	0.0	0.5
14	11,200	52.0	35.5	7.5	3.0	0.5	1.5	0.0

SERIES I (CONTINUED).

Age 2 to 3 Years.

Third Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{15}{15}$ 23,700	63.5	24.5	0.0	9.5	0.0	2.0	0.5
5	$\frac{19}{19}$ 13,600	45.0	42.5	5.0	6.0	0.0	1.0	0.5
6	$\frac{19}{19}$ 12,800	55.0	16.0	24.5	2.5	0.5	1.5	0.0
8	$\frac{15}{15}$ 14,100	52.0	27.5	14.5	2.5	0.0	2.5	0.5
15	$\frac{19}{15}$ 18,700	47.5	35.5	7.0	7.0	0.0	3.0	0.0

Fourth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{22}{22}$ 23,600	65.0	23.0	4.0	7.0	0.0	1.0	0.0
6	$\frac{26}{31}$ 18,800	43.5	46.0	0.5	10.0	0.0	0.0	0.0
8	$\frac{27}{26}$ 14,600	26.5	50.0	10.5	7.0	0.0	6.0	0.0
10	$\frac{24}{23}$ 19,800	61.0	23.5	9.5	4.5	0.5	0.5	0.0
11	$\frac{24}{23}$ 23,000	57.0	32.0	0.0	7.5	0.0	3.0	0.0

Fifth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{29}{29}$ 19,400	46.0	33.5	4.5	6.0	0.0	9.5	0.5
6	$\frac{34}{36}$ 21,700	57.5	36.0	2.0	5.0	0.0	2.5	0.0
7	$\frac{30}{28}$ 16,400	40.0	38.5	9.5	14.0	0.0	8.0	0.0
8	$\frac{34}{35}$ 15,000	43.0	44.5	5.0	3.5	0.0	3.5	0.0
9	$\frac{30}{34}$ 18,100	58.0	21.5	5.5	11.5	0.0	3.5	0.0
11	$\frac{33}{32}$ 27,000	46.0	41.0	0.0	5.0	0.0	7.5	0.0

Sixth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{38}{38}$ 22,800	57.0	29.0	4.5	4.5	0.5	2.5	2.0
6	$\frac{42}{37}$ 14,800	51.5	44.0	3.0	0.5	0.0	0.5	0.5
9	$\frac{36}{30}$ 10,200	45.0	39.0	2.0	9.0	0.0	3.5	1.0

Seventh Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{46}{46}$ 15,800	70.0	16.0	9.5	3.5	0.0	1.0	0.0
6	$\frac{49}{44}$ 22,300	42.5	43.6	0.5	7.5	0.5	1.0	0.0
9	$\frac{43}{37}$ 16,800	51.5	25.5	11.0	6.5	0.5	5.0	0.0

SERIES I (CONTINUED).

Age 3 to 4 Years.

First Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{4}{1}$ 6,900	56.5	29.0	—	13.0	0.5	0.0	1.0
	$\frac{6}{3}$ 8,300	72.0	21.5	1.0	5.0	0.0	0.0	0.5
2	$\frac{6}{2}$ 15,400	63.0	17.0	5.0	8.5	0.0	6.0	0.5
3	$\frac{3}{1}$ 10,100	61.0	26.5	6.5	5.0	0.0	0.5	0.5
4	$\frac{7}{1}$ 15,100	55.0	26.0	8.5	1.5	0.0	9.0	0.0

Second Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{13}{10}$ 14,600	57.5	21.5	13.5	6.5	0.0	0.5	0.5
5	$\frac{14}{11}$ 19,700	84.0	6.5	6.5	3.0	0.0	0.0	0.0

Third Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{20}{17}$ 10,300	59.5	20.0	6.5	14.0	0.0	0.5	0.0
4	$\frac{17}{16}$ 15,700	45.0	37.5	2.0	5.5	1.0	6.5	—

Fourth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{24}{23}$ 19,800	51.0	37.5	0.0	3.5	0.0	8.0	0.0
5	$\frac{26}{23}$ 11,000	40.5	46.5	1.5	8.5	0.5	2.0	0.5

Fifth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{29}{25}$ 12,800	54.0	30.0	6.0	6.5	0.0	2.5	0.5
4	$\frac{33}{32}$ 16,700	55.0	30.0	10.5	2.5	0.0	2.0	0.0
5	$\frac{34}{32}$ 15,000	60.5	25.0	6.5	3.0	1.0	4.0	0.0

Seventh Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{36}{33}$ 9,800	49.5	30.5	4.0	11.5	0.5	4.0	0.0

SERIES I (CONTINUED).

Age 4 to 5 Years.

First Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	33,000	——	——	——	——	—	—	—
4	19,300	73.0	18.6	2.6	4.6	0.0	0.0	0.6
5	17,600	44.5	37.5	3.0	14.5	0.0	0.0	0.5
6	20,000	49.0	25.0	26.5	8.0	1.5	0.0	0.0
7	13,000	51.5	37.0	7.0	4.5	0.0	0.0	0.0
8	12,400	53.0	24.0	9.5	12.0	0.0	0.0	0.0

Second Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	28,000	——	——	——	——	—	—	—

Third Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles	Eosinophiles.	Stimulation forms.
3	20,800	——	——	——	——	—	—	—

Fourth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	10,500	55.0	30.0	10.5	4.0	0.0	0.5	0.0
2	12,000	58.5	27.9	9.0	2.0	0.0	3.5	0.0
3	22,300	42.0	33.8	6.4	2.1	0.6	2.7	0.3

Fifth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
2	14,800	85.0	——	——	——	—	—	—
	6,300	62.5	19.0	12.0	3.5	1.0	2.0	0.0
3	20,000	65.0	24.0	0.5	7.0	0.0	2.0	1.0

Sixth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	13,100	64.0	23.5	8.5	3.0	0.0	1.0	0.0
3	28,600	57.5	34.0	7.0	1.5	0.0	0.0	0.0

Seventh Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	12,400	39.5	54.5	0.5	4.0	0.5	0.5	0.5
2	11,000	62.0	16.0	10.5	0.5	9.5	1.0	0.5

SERIES I (CONTINUED).

Age 4 to 5 Years.

Eighth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	12,000	47.0	37.5	8.5	5.5	0.0	1.5	0.0
2	10,700	52.5	35.5	9.0	2.5	0.0	0.5	0.0

Tenth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	10,800	61.5	23.5	4.5	9.0	0.0	0.0	0.5

Twelfth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	16,000	54.0	38.0	5.5	2.0	0.0	1.5	0.0

Age 5 to 7 Years.

First Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	19,400	62.2	16.7	11.7	1.0	0.2	8.0	0.0
2	23,200	76.0	17.0	3.0	2.5	1.0	0.0	0.5
3	8,800	77.0	17.5	0.5	4.0	0.0	0.0	1.0
4	20,800	66.0	24.0	1.0	5.5	1.5	2.0	0.0

Second Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
3	11,800	76.5	14.5	4.5	3.5	0.0	0.5	0.5

Third Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	28,800	72.0	9.0	2.0	10.0	0.0	2.0	0.0

Fourth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	14,200	49.5	36.0	12.5	1.5	0.0	0.0	0.0
2	21,100	64.5	25.0	7.5	2.5	0.5	0.0	0.0

SERIES I (CONCLUDED).

Age 5 to 7 Years.

Sixth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{17}{11}$ 15,600	50.0	32.5	4.0	11.0	0.0	1.5	1.0

Seventh Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{44}{11}$ 16,000	59.5	22.0	7.5	8.5	0.5	2.0	0.0

Age 8 to 12 Years.

First Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
2	$\frac{7}{1}$ 16,700	75.0	15.0	0.5	9.5	0.0	0.0	0.0
3	$\frac{4}{2}$ 12,000	75.0	13.0	3.5	8.0	0.0	1.0	0.0
4*	$\frac{5}{3}$ 13,000	59.0	17.5	17.0	4.5	0.5	1.5	0.0

Second Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophilos.	Stimulation forms.
4	$\frac{13}{11}$ 12,000	62.0	17.5	16.0	3.5	0.0	1.0	0.0

Third Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{18}{11}$ 7,800	63.0	28.0	4.5	2.0	0.0	2.5	0.0
4	$\frac{21}{19}$ 18,000	67.5	19.5	3.5	9.0	0.0	1.0	0.0

Fourth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{28}{21}$ 9,400	64.5	26.0	0.0	4.5	0.5	4.5	0.0
4	$\frac{27}{25}$ 10,700	62.5	26.0	2.5	8.0	0.0	0.5	0.5

Sixth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1	$\frac{42}{35}$ 9,400	47.0	37.5	5.5	4.0	0.0	3.5	2.5

Eighth Week.

Number of case.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4	$\frac{52}{50}$ 16,000	58.0	32.5	1.0	6.0	0.0	2.0	0.5

* Age, 21 years.

SERIES II.

First Week.

Age.	Number of cases.	Total leukocyte count.		Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
6–12 mos.	4	23,900–35,600	Maximum	59.0	57.0	18.0	15.0	0.5	1.0	1.5
			Minimum	25.0	16.5	1.0	0.5	0.0	0.0	0.0
			Average	42.0	36.7	9.5	7.7			
1–2 yrs.	12	12,200–26,000	Maximum	70.0	50.0	13.5	13.5	1.0	15.5	1.0
			Minimum	26.5	16.5	1.5	2.5	0.0	0.0	0.0
			Average	48.2	33.2	7.5	8.0			
2–3 yrs.	5	9,000–23,000	Maximum	64.5	43.0	14.0	18.0	1.5	7.0	1.5
			Minimum	32.5	20.0	1.5	3.0	0.0	0.0	0.0
			Average	48.5	31.5	7.7	10.5			
3–4 yrs.	4	6,900–15,000	Maximum	72.0	29.0	8.5	13.0	0.5	9.0	1.0
			Minimum	55.0	17.0	1.0	1.5	0.0	0.0	0.0
			Average	68.5	23.0	4.7	7.2			
4–5 yrs.	6	12,400–33,000	Maximum	81.0	37.5	26.5	14.5	1.5	0.0	0.5
			Minimum	44.5	18.6	2.6	4.5	0.0	0.0	0.0
			Average	62.7	28.0	14.5	9.5			
5–7 yrs.	4	8,800–23,300	Maximum	77.0	24.0	11.7	5.5	1.0	8.0	1.0
			Minimum	62.2	16.7	0.5	1.0	0.0	0.0	0.0
			Average	69.6	20.3	6.5	3.2			
8–21 yrs.	3	12,000–16,700	Maximum	75.0	17.5	17.0	9.5	0.05	1.5	0.0
			Minimum	59.0	13.0	0.5	4.5	0.0	0.0	0.0
			Average	67.0	15.2	8.7	7.0			
Total	38									

Second Week.

Age.	Number of cases.	Total leukocyte count.		Polymorphonuclears	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1–2 yrs.	11	12,300–30,400	Maximum	73.0	56.0	23.0	11.0	6.0	5.5	0.05
			Minimum	37.0	9.0	0.5	4.0	0.0	0.0	0.0
			Average	55.0	32.5	11.7	7.7			
2–3 yrs.	8	9,800–20,000	Maximum	61.5	41.0	23.5	10.5	5.0	7.5	0.5
			Minimum	38.0	10.0	2.0	2.0	0.0	0.0	0.0
			Average	49.7	25.5	12.7	6.2			
3–4 yrs.	2	14,600–19,700	Maximum	84.0	21.5	13.5	6.5	0.0	0.5	0.5
			Minimum	57.5	6.5	6.5	3.0	0.0	0.0	0.0
			Average	70.7	14.0	10.0	4.7			
4–5 yrs.	1	28,000								
5–7 yrs.	1	11,800		76.5	14.5	4.5	3.5	0.0	0.5	0.5
8–12 yrs.	1	12,000		62.0	17.5	16.0	3.5	0.0	1.0	0.0
Total	24									

SERIES II (CONTINUED).

Third Week.

Age.	Number of cases.	Total leukocyte count.		Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1–2 yrs.	13	8,300–26,100	Maximum	67.0	58.5	7.5	13.5	1.5	10.5	1.0
			Minimum	30.5	22.0	0.0	0.5	0.0	0.0	0.0
			Average	48.7	40.2	3.7	7.0			
2–3 yrs.	5	12,800–23,700	Maximum	63.5	42.5	24.5	9.5	0.5	3.0	0.5
			Minimum	45.0	16.0	0.0	2.5	0.0	1.0	0.0
			Average	54.7	29.2	12.2	6.0			
3–4 yrs.	2	10,300–15,700	Maximum	59.5	37.5	6.5	14.0	1.0	6.5	0.0
			Minimum	45.0	20.0	2.0	5.5	0.0	0.5	0.0
			Average	52.2	28.7	4.2	9.7			
4–5 yrs.	1	28,000								
5–7 yrs.	1	28,000		72.0	9.0	2.0	10.0	0.0	2.0	0.0
8–12 yrs.	2	7,800–18,000	Maximum	67.5	28.0	4.5	9.0	0.0	2.5	0.0
			Minimum	63.0	19.5	3.5	2.0	0.0	1.0	0.0
			Average	65.2	23.7	4.0				
Total	24									

Fourth Week.

Age.	Number of cases.	Total leukocyte count.		Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1–2 yrs.	11	10,100–24,700	Maximum	60.0	63.5	41.0	17.0	1.0	4.0	2.5
			Minimum	26.5	21.5	35.0	1.5	0.0	0.0	0.0
			Average	43.2	42.5	23.7	9.5			
2–3 yrs.	5	14,600–23,600	Maximum	65.0	46.0	10.5	10.0	0.5	6.0	0.0
			Minimum	26.5	23.5	0.5	4.5	0.0	0.0	0.0
			Average	45.7	34.7	5.5	7.2			
3–4 yrs.	2	11,000–19,800	Maximum	51.0	46.5	1.5	8.5	0.5	8.0	0.5
			Minimum	40.5	37.5	0.0	3.5	0.0	2.0	0.0
			Average	45.7	42.0	0.7	6.0			
4–5 yrs.	3	10,500–22,300	Maximum	58.5	33.8	10.5	4.0	0.6	2.7	0.3
			Minimum	42.0	27.9	6.4	2.0	0.0	0.5	0.0
			Average	50.2	30.8	8.4	3.0			
5–7 yrs.	2	14,200–21,000	Maximum	64.5	36.0	12.5	2.5	0.5	0.0	0.0
			Minimum	49.5	25.0	7.5	1.5	0.0	0.0	0.0
			Average	57.0	30.0	10.0	2.0			
8–12 yrs.	2	9,400–10,700	Maximum	64.5	26.0	2.5	8.0	0.5	4.5	0.5
			Minimum	62.5		0.0	4.5	0.0	0.5	0.0
			Average	63.5		1.7	6.2			
Total	25									

SERIES II (CONTINUED).

Fifth Week.

Age.	Number of cases.	Total leukocyte count.		Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1–2 yrs.	6	9,100–23,600	Maximum	60.5	56.0	13.5	10.0	0.5	6.5	1.0
			Minimum	38.0	20.5	1.0	2.0	0.0	1.5	0.0
			Average	49.2	38.2	7.2	6.0			
2–3 yrs.	6	15,000–27,000	Maximum	58.0	44.5	9.5	14.0	0.0	9.5	0.5
			Minimum	40.0	21.5	0.0	5.0	0.0	3.5	0.0
			Average	49.0	33.0	4.2	9.5			
3–4 yrs.	3	12,800–16,700	Maximum	60.5	30.0	10.5	6.5	1.0	4.0	0.5
			Minimum	54.5	25.0	6.0	2.5	0.0	2.0	0.0
			Average	57.5	27.5	8.2	4.5			
4–5 yrs.	2	6,300–20,000	Maximum	85.0	24.0	12.0	7.0	1.0	2.0	1.0
			Minimum	62.5	19.0	0.5	3.5	0.0		0.0
			Average	73.7	21.5	6.2	5.2			
Total	17									

Sixth Week.

Age.	Number of cases.	Total leukocyte count.		Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1–2 yrs.	3	9,600–26,700	Maximum	40.0	69.0	10.0	15.0	0.5	2.5	2.0
			Minimum	23.0	32.0	0.0	0.5	0.0	0.5	0.0
			Average	31.5	50.5	5.0	7.7			
2–3 yrs.	2	10,200–22,800	Maximum	57.0	44.0	4.5	9.0	0.5	3.5	2.0
			Minimum	45.0	29.0	2.0	0.5	0.0	0.5	0.5
			Average	51.0	36.5	3.2	4.7			
4–5 yrs.	2	13,100–28,600	Maximum	64.0	34.0	8.5	3.0	0.0	1.0	0.0
			Minimum	57.5	23.5	7.0	1.5	0.0	0.0	0.0
			Average	60.7	28.7	7.7	2.2			
5–7 yrs.	1	15,000		50.0	32.5	4.0	11.0	0.0	1.5	1.0
8–12 yrs.	1	9,400		47.0	37.5	5.5	4.0	0.0	3.5	2.5
Total	9									

SERIES II (CONCLUDED).

Seventh Week.

Age.	Number of cases.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
1–2 yrs.	2	23,700–26,700	Maximum 51.0	45.0	10.0	5.0	2.0	6.5	0.5
			Minimum 31.5	34.5	9.5	4.0	0.0	0.5	0.0
			Average 41.2	39.7	9.7				
2–3 yrs.	3	15,800–22,300	Maximum 70.0	43.6	11.0	7.5	0.5	5.0	0.0
			Minimum 42.5	16.0	0.5	3.5	0.0	1.0	0.0
			Average 56.2	29.8	5.7	5.5			
3–4 yrs.	1	9,800	49.0	30.5	4.0	11.5	0.5	4.0	0.0
4–5 yrs.	2	11,000–12,400	Maximum 62.0	54.5	10.5	4.0	9.5	1.0	0.5
			Minimum 39.5	16.0	0.5	0.5	0.5	0.5	
			Average 50.7	35.2	5.5	2.2			
5–7 yrs.	1	16,000	59.5	22.0	7.5	8.5	0.5	2.0	0.0
Total	9								

Eighth Week.

Age.	Number of cases.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4–5 yrs.	2	10,700–12,000	Maximum 52.5	37.5	9.0	5.5	0.0	1.5	0.0
			Minimum 47.0	35.5	8.5	2.5	0.0	0.5	
			Average 49.7	36.5	8.7	4.0			
8–12 yrs.	1	16,000	58.0	32.5	1.0	6.0	0.0	2.0	0.5
Total	3								

Tenth Week.

Age.	Number of cases.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4–5 yrs.	1	10,800	61.5	23.5	4.5	9.0	0.0	0.0	0.5

Twelfth Week.

Age.	Number of cases.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
4–5 yrs.	1	16,000	54.0	38.0	5.5	2.0	0.0	1.5	0.0

ALL AGES.

	Number of cases.	Leukocyte count.		
		Maximum.	Minimum.	Average.
1st week	38	24,600	12,200	18,400
2d week	24	24,500	12,000	18,250
3d week	24	23,300	10,400	16,800
4th week	25	20,300	11,600	15,900
5th week	17	21,800	10,800	16,300
6th week	9	23,200	10,500	16,800
7th week	9	19,500	15,000	17,250
8th week	3	14,000	10,700	12,400
10th week	1			10,800
12th week	1			16,000
Total	151			

The following table comprises separately the counts from preparalytic and abortive cases.

	Age.	Total leukocyte count.	Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Transitionals.	Basophiles.	Eosinophiles.	Stimulation forms.
Breed's case		15,000	—	—	—	—	—	—	—
		7,000	81.0	—	—	—	—	—	—
J. S.	2½ yrs.	14,200	58.5	31.5	7.0	3.0	0.0	0.0	0.0
C. T.	8 mos.	35,600	32.0	55.0	1.0	9.0	0.0	1.0	1.5
E. N.	6 yrs.	23,200	76.0	17.0	3.0	2.5	0.0	1.0	0.5
H. B.	6 yrs.	8,800	77.0	17.5	0.5	4.0	0.0	0.0	1.0
		11,200	79.5	14.5	0.5	4.5	0.0	0.5	0.5
		5,600	68.5	22.0	1.5	6.0	0.0	1.0	1.0
		11,800	76.5	14.5	4.5	3.5	0.0	0.5	0.5
P. T.	2 yrs.	15,000	52.5	31.5	13.5	1.5	0.5	0.5	0.0

It is obvious from these figures that while in most cases in the preparalytic stage the total leukocyte count varies within the normal, there is a tendency toward the upper limits. More cases showed counts well above than below the usual extremes. In the differential counts there is a definite polymorphonucleosis with the one exception of a count in an infant eight months old, where the relation of polymorphonuclears and lymphocytes is normal. Furthermore, the lymphocyte percentages in all the other cases are distinctly below the usual figure.

In one instance where blood counts were made by the physician before the patient came to the hospital, a low total count appeared;

7,000, two days before the paralysis, and 3,000 on the day after paralysis. The differential count, however, showed a polymorphonuclear increase each time (81 per cent. and 58 per cent.). Except for this one case which subsequently became one of our seventy-one, we have not seen a true leukopenia. One abortive case in a little girl six years of age showed counts of 5,600 to 11,800. In the youngest children the leukocyte counts have been highest, and the polymorphonuclear increase both relatively and actually has been more marked than in the older patients. A glance at the tables, however, will show that all ages have a high white cell count. Furthermore, the leukocytosis persists for a surprising length of time. In nine cases, for example, observed during the seventh week, the leukocytes numbered 15,000 to 19,500, with an average of 17,250.

The differential count presents a fairly constant picture. For comparison, a table of differential counts from normal individuals prepared by Schloss[41] is printed. There is obviously in poliomyelitis an increase in polymorphonuclear cells of 10 to 15 per cent. above the normal. This increase appears irrespective of the patient's age, though the youngest children have shown perhaps the highest polymorphonuclear counts. Contrary to the observations of Müller, and Gay and Lucas, we have found a diminution of lymphocytes. In general, they have been from 15 to 20 per cent. below the normal percentages. Notwithstanding the great variation which these cells present in relation to age, the finding of low lymphocyte percentages has been constant in all our cases of poliomyelitis.

The transitional and large mononuclear cells have reached no abnormal figures. In many cases there has been an increase in eosinophiles. This finding, however, has not been sufficiently constant to justify further comment. No counts were made of the red cells, nor were any hemoglobin estimations recorded. There has been nothing in the appearance of the patients to suggest an anemia resulting from the disease; nor did the stained erythrocytes look abnormal. No actual counts of the blood platelets were made, but the impression was gained in the course of many differential counts that these elements were somewhat increased.

[41] Schloss, *Arch. Int. Med.*, 1910, vi, 638.

DIFFERENTIAL BLOOD COUNTS IN NORMAL INDIVIDUALS.

Age.		Polymorphonuclears.	Lymphocytes.	Large mononuclears.	Eosinophiles.	Basophiles.
6–12 mos.	Maximum	35.9	58.5	12.2	4.5	0.8
	Minimum	24.6	50.5	7.3	0.0	0.1
	Average	30.4	55.9	9.6	2.6	0.4
1–2 yrs.	Maximum	39.7	58.8	11.7	6.0	0.5
	Minimum	27.5	45.3	6.7	1.6	0.0
	Average	36.3	51.2	8.5	3.2	0.2
2–3 yrs.	Maximum	44.3	55.0	11.3	6.0	1.2
	Minimum	33.2	43.5	5.0	0.5	0.0
	Average	38.7	49.0	8.2	3.1	0.4
3–4 yrs.	Maximum	54.1	47.6	16.2	4.2	0.9
	Minimum	36.2	32.2	5.0	1.5	0.0
	Average	44.7	39.1	11.2	2.8	0.5
4–5 yrs.	Maximum	51.7	49.5	6.7	4.0	0.6
	Minimum	42.2	38.4	3.4	1.6	0.3
	Average	48.5	42.1	6.0	2.6	0.3
5–6 yrs.	Maximum	61.8	36.7	16.0	4.7	1.0
	Minimum	52.6	21.2	6.5	0.7	0.3
	Average	56.5	29.9	10.0	2.5	0.6
6–7 yrs.	Maximum	61.3	34.1	15.7	4.7	0.6
	Minimum	52.3	24.5	8.1	0.1	0.0
	Average	56.0	30.4	10.8	2.2	0.2
7–8 yrs.	Maximum	72.0	39.1	15.2	3.5	0.2
	Minimum	45.2	21.1	6.7	0.0	0.0
	Average	54.4	32.5	11.6	0.1	0.06

To sum up, we have found in the blood of patients with poliomyelitis a constant and marked leukocytosis. In several instances the count has been as high as 30,000. In only one case has there been a definite leukopenia. Besides the increase in the total number of cells, there has been an equally constant increase of polymorphonuclears of 10 to 15 per cent., and diminution of lymphocytes of 15 to 20 per cent. The other forms of leukocytes have shown no abnormalities.

The recent demonstration of the visceral lesions of poliomyelitis, showing the active part of phagocytosis played by the polymorphonuclear cells is perhaps sufficient cause for the polymorphonuclear increase in the circulating blood. In view of the fact, however, that the brunt of the body's attempt at defense falls upon the

lymphatic system, and that the cells which are assembled at the point of attack seem to be lymphocytes, one might be led at first to expect a lymphocytosis in the blood. On the other hand, the great destruction of lymphocytes, which is evident in the visceral lesions, suggests that these cells are relatively few in the circulation because they are constantly being withdrawn to meet the invasion of the virus at its various points of attack. It will require a more fundamental knowledge of the kind of reaction the body develops to this type of infecting agent before a correct explanation of the blood picture can be made. At all events, while the blood picture in poliomyelitis is perhaps not any more specific than is the spinal fluid, it is helpful. If taken in connection with other available evidence, a leukocytosis of 15,000 to 30,000 is distinctly suggestive of the disease in question, especially if the polymorphonuclear cells are increased at the expense of the lymphocytes.

THE CEREBROSPINAL FLUID.

There are two ways in which a careful study of the cerebrospinal fluid in acute poliomyelitis may help to throw light on the disease. On the one hand, the changes observed may facilitate early diagnosis and thus enhance the value of any method of treatment which may be discovered in the future, and, on the other hand, the variations in the character of the fluid, as observed during the progress of the disease in each case, may give some information as to the usual course of the process and thus be an aid in determining how far any given remedy is effective or to what extent the natural course of the disease may be influenced. The following study was thus made with the twofold object of determining whether the examination of the spinal fluid may be an aid in early diagnosis, and what changes occur in the fluid during the course of the disease.

Previous investigations along this line have not been extensive. Most writers report only a few cases, and those who have apparently made a large number of lumbar punctures have not submitted the fluids to careful examination. The results obtained are somewhat variable, but in general the fluid is said to contain an abnormal amount of albumen and a sediment consisting of lymphocytes, large mononuclear cells, and, occasionally, polymorphonuclear cells. Im-

portant work has been done by Gay and Lucas,[42] whose contributions are the most valuable in this field. Their studies have included the spinal fluids from monkeys in the incubation period, the prodromal stage, and the acute stage, and from eleven human cases in the preparalytic stage. Besides noting the character of the cells, they have estimated the number of cells per cubic millimeter. In the eleven early cases, the cell count varied from 55 to 580 per cubic millimeter, and the percentage of mononuclears from 75 to 100. Chemical examinations were omitted. Sophian[43] reports that the globulin test is positive in the early stages.

Inasmuch as previous work by Wollstein,[44] Römer and Joseph,[45] and Gay and Lucas failed to demonstrate the presence of any specific antibody in the spinal fluid in infantile paralysis, we have confined our study to simple chemical and cytological examinations.

It has been our object to see the cases as early as possible after the onset of the disease, to make a lumbar puncture on admission to the hospital, to repeat the puncture every two to four days in the early stages, and in the later stages to make a puncture every five to ten days while the case was under observation, or until the fluid became normal. We have examined in all 233 fluids from sixty-nine cases. The number of cases seen in the first week of the disease (dating from onset of symptoms, not of paralysis) was forty-three, in the second week, forty-five, in the third week, forty, and in the fourth week, thirty. The number of cases seen later in the course was much smaller. It is to be regretted that the number of preparalytic and abortive cases was so small; of the former, four, and of the latter only two were seen in the active stage. It was, however, rare that cases were referred to the hospital until a positive diagnosis, based on the appearance of paralysis, had been made by the attending physician.

The methods used for the examination of the cerebrospinal fluid have included estimation of the pressure, determination of the number of cells per cubic millimeter and the types of cells present,

[42] Gay and Lucas, *Arch. Int. Med.*, 1910, vi, 330; Lucas, *Am. Jour. Dis. Child.*, 1911, i, 230.

[43] Sophian, *Arch. Pediat.*, 1911, xxviii, 201.

[44] Wollstein, *Jour. Exper. Med.*, 1908, x, 476.

[45] Römer and Joseph, *München. med. Wchnschr.*, 1910, lvii, 568.

estimation of the chlorides, and observations of the power of reducing Fehling's solution, and the globulin content. Globulin was tested by the butyric acid method of Noguchi, and the relative strength of the reaction noted. Thus very slightly plus (v. s. +) indicates a faint precipitate, which is, however, definitely more than that obtained with normal spinal fluids; slightly plus (s. +) is the term applied to reactions giving a well marked cloud or a slightly flocculent precipitate; plus (+) signifies a precipitate coming down in large flocculi; and double plus (++), a heavy flocculent precipitate. The reaction was performed with accurately measured amounts of spinal fluid and of reagents, and the reading was made after five to ten minutes' standing.[46] The gross appearance of the fluids was very constant. Almost all were clear, colorless, and watery. Only a few showed the slightest opalescence. On standing, a delicate web-like clot formed in a small proportion of cases. Clot formation was most frequently seen in the early stages of the disease. It bore no apparent relation to the globulin content of the fluid.

The results obtained by several of these methods may be dismissed briefly. Pressure determinations were made in a number of cases and readings were usually above normal. The pressure was read in millimeters of spinal fluid in a tube of one millimeter bore. No extremely high readings were obtained, and in some instances normal pressures were seen during the early stages of the disease. On the whole, the estimations of pressure were unsatisfactory, as the patients were usually crying children.

The chlorides were estimated in a series of cases with the idea that they might throw light on the inorganic substances in the fluid. No marked deviations from the normal were observed. Every fluid examined showed a power of reducing Fehling's solution which was, roughly speaking, normal. This reaction serves as a point of differentiation from many fluids in cases of meningitis, in which the reducing substance may be absent.

The type of cell present in the fluid was almost always the

[46] To 0.2 c.c. of spinal fluid is added 0.5 c.c. of butyric acid solution (10 per cent. butyric acid in 0.85 per cent. solution of sodium chloride). The mixture is boiled, 0.1 c.c. of normal sodium hydrate solution added, and then boiled again.

mononuclear. A large number of differential counts showed that the relative number of the different types of mononuclear cells was of comparatively little importance, so in later observations we were content with noting merely the relative number of mononuclear and polymorphonuclear cells. In several instances numbers of very large phagocytic cells containing vacuoles and broken down cells were seen. Once this occurred several weeks after the onset of the disease in association with a sudden rise in the cell count resembling that seen in acute meningitis. Polymorphonuclear cells, except in small number and probably due for the most part to a slight admixture of blood, were, except in a very few instances, absent after the first week of the disease. Early in the disease, however, and in the preparalytic period, the polymorphonuclears sometimes outnumbered the mononuclears and made up 80 to 90 per cent. of the total.

The results described by Flexner and Lewis[47] of a study of the cerebrospinal fluid of a monkey after inoculation with the virus of poliomyelitis are interesting. Twenty-four hours after inoculation the fluid contained "a considerable number of small cells, hardly exceeding a lymphocyte in size but showing a polyform nucleus, a few lymphocytes and some red corpuscles. At the expiration of forty-eight hours, the white cells have increased in numbers, but the cells with polyform nuclei still predominate. At the expiration of seventy-two hours, a large number of mononuclear cells have appeared and the fluid presents a striking opalescent appearance. On the day of paralysis the fluid tends to be only slightly cloudy and contains a mixture of large and smaller (lymphoid) mononuclear cells and a few cells with polymorphous nuclei." Lucas, too, found in the monkey during the prodromal stage "a marked increase in the cells, often reaching 1,000 per cubic millimeter. In this stage, also, polynuclears are still present, in some cases as high as 60 per cent., though the large mononuclears and lymphocytes were very evident. In the early acute stage the increase in cells is very marked. The cells are now, however, mostly of the lymphocytic or very early form of cells, and sometimes very hard to place, as they are apparently undifferentiated cells." Of his eleven preparalytic

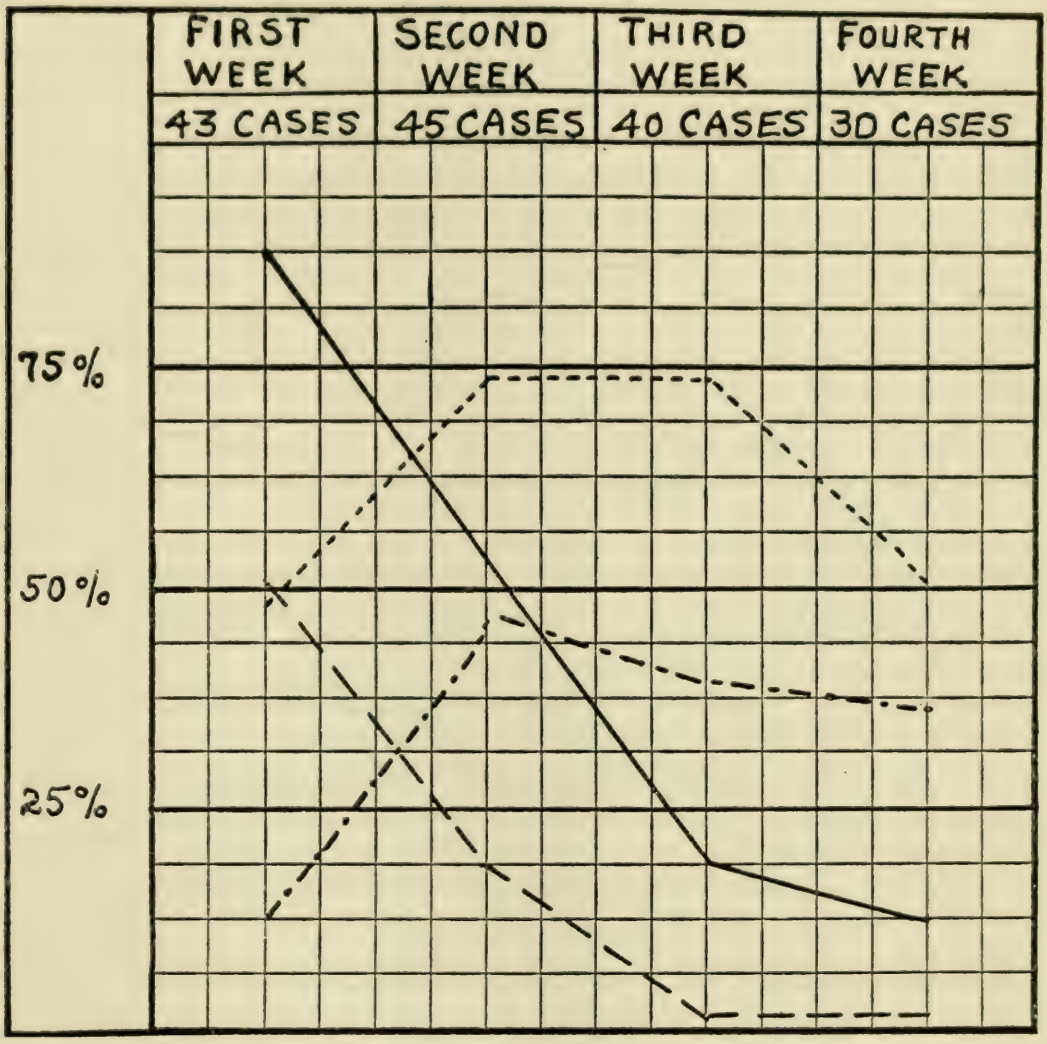
[47] Flexner and Lewis, *Jour. Am. Med. Assn.*, 1910, liv, 1140.

human cases, however, only one showed less than 87 per cent. of mononuclears, and that had 75 per cent. of mononuclears. A return of polymorphonuclears later in the disease, as was reported by Lucas to occur in monkeys, was not noticeable in our human cases.

The cell count was made immediately after the fluid was obtained. The first portion of fluid obtained was used, but controls made by comparing counts of early and late portions of the fluid showed no important discrepancy. While the normal number of cells per cubic millimeter of spinal fluid is usually given as 3 to 5, we have considered the upper normal limit as 10 to 12. In general, it may be said that the highest cell counts are found in the early days of the disease, and that there is a progressive falling off as time advances. Thus of forty-three cases in the first week, twenty-three showed fluids with over fifty cells per cubic millimeter, and thirty-eight with cell counts above normal. Two cases showed in the prodromal period 990 and 650 cells respectively. Of forty-five cases in the second week, in eight the counts were over fifty, and in twenty-three cases they were above normal; of forty cases in the third week in only one was the count over fifty, and in only eight above normal. On the other hand, six cases in the first week, twenty-two cases in the second week, and thirty-two of the forty cases in the third week showed normal counts.

In contrast to the cell count which thus tends to be high in the early part of the disease, the globulin content is usually low in the first part of the acute stage, especially as compared with the cell count. It rises during the second and third weeks and then gradually falls, though frequently globulin is present long after all acute symptoms have passed (text-figure 2). We have seen a strong + globulin reaction on the forty-sixth day of the disease. During the first week, only six of forty-three cases showed at any time a globulin reaction of + (see above) or more. During the second week, twenty out of forty-five cases showed a + reaction; in the third week sixteen out of forty, and in the fourth week nine out of thirty. On the other hand, sixteen cases in the first week showed an absolutely normal globulin reaction. Five cases in the second week and only three in the third week were negative for globulin. Most cases, even on discharge, gave a globulin reaction which varied from

slightly to markedly above the normal. In eleven cases the fluids were followed until both cell count and globulin reaction became normal. The return to normal occurred in the third week in two cases; in the fourth, in two cases; in the fifth, in one case; in the



TEXT-FIG. 2. Chart showing variations in cell count and in globulin content of cerebrospinal fluid in infantile paralysis. ——— Percentage of cases with cell count above normal. — — — Percentage of cases with cell count above 50 per c.mm. - - - - Percentage of cases with globulin s. +. —·—·— Percentage of cases with globulin + or over.

sixth, in two cases; in the seventh, in three cases; and in the tenth week, in one case.

Of the sixty-nine cases studied, only two never showed any abnormal fluids. In each of these cases the fluids were examined but

once, on the twenty-fifth and thirty-third days of the disease respectively. One case showed, in five fluids taken between the ninth and forty-first days of the disease, no evidence of abnormality other than the slightest positive globulin reaction (v. s. +), and another, in three fluids obtained on the ninth, sixteenth, and twenty-third days, gave similar results. Four cases, including one abortive case, showed fluids which, except for the slightest globulin reaction, were normal at the first tapping and subsequently became more definitely positive.

The commonest type of fluid seen in the whole series is one with a normal or slightly increased cell count, and a well marked globulin reaction. There are, however, two other distinct types of fluid seen. One is a fluid with a high cell count, and a normal or very slight globulin reaction; and the other is a fluid with a normal or low cell count, and a very marked globulin reaction. Twenty-six cases showed at some time a globulin reaction of not over s. +, and a cell count of fifty or over. Twenty of these occurred in the first week, and in twelve the count was over 100 cells per cubic millimeter. On the other hand, sixty-two fluids from thirty-four cases gave a globulin reaction of + or more. In thirty-three of these the cell count was normal, and in fifty-five it was not above forty per cubic millimeter. That the two types of fluid are fairly distinct is shown by the fact that only five cases showed fluids with both a high cell count (over fifty) and even a moderately high globulin reaction. Each type of fluid is, moreover, quite definitely characteristic of a stage in the disease. The cellular exudate is almost always associated with the earliest days of the acute stage; the albuminous exudate, with the latter part of the acute stage. It is interesting that of ten cases seen within the first three days after the onset of symptoms, all but one had a cell count of over fifty (one was thirty-seven), and six were over 100. On the other hand, six gave a negative globulin reaction. In four the reaction was s. +.

As regards early diagnosis, the cases examined before the onset of paralysis are of the greatest interest. Six cases were seen in what may be termed the prodromal period. Four of these developed paralysis later and two proved to be of the abortive type. In one case a single lumbar puncture was made on the fourth day

after the onset. The fluid gave a cell count of 650 per cubic millimeter, 90 per cent. polynuclears, and a + globulin reaction. Three days later the child suddenly developed paralysis and in five hours died of respiratory failure. A second case, the sister of a child already in the hospital, was seen on the day after the onset of symptoms. The spinal fluid contained 990 cells per cubic millimeter, 90 per cent. of which were polymorphonuclears, and the globulin reaction was s. +. On the following day one leg was paralyzed and the cell count of the spinal fluid was 627 per cubic millimeter with 80 per cent. mononuclears. On the next day both legs were paralyzed and the cell count was 1,221 per cubic millimeter with 92 per cent. mononuclears, including many large phagocytic cells. The globulin reaction was + (case 34, page 184). Thus two cases seen very early in the course of the disease showed fluids with unusually high cell counts, and with a marked predominance of polymorphonuclear cells. In one of them the change of the cell picture from the polynuclear type to the mononuclear type could be followed coincidentally with the onset of the paralysis. Three other cases in the prodromal period, one an abortive case and two becoming paralyzed on the day of the first lumbar puncture, gave cell counts of 37, 94, and 113 per cubic millimeter, with 100 per cent., 93 per cent., and 83 per cent. of mononuclear cells respectively, and globulin reactions which fell within normal limits. Finally, in one abortive case lumbar puncture on the second day after the onset of symptoms gave a wholly normal fluid. On the third day the cell count was sixty-two per cubic millimeter with 89 per cent. mononuclears and a s. + globulin reaction. On the sixth day the cell count was fifty-nine per cubic millimeter, all mononuclears, and a globulin reaction of s. +. On the eleventh day the spinal fluid was again normal. That this was, indeed, an abortive case of poliomyelitis was later rendered most probable by a test showing that the patient's blood serum was capable of neutralizing active virus when mixed with it and injected intracranially into a monkey.

In these six cases examination of the spinal fluid gave evidence which was helpful in making a diagnosis of poliomyelitis before the onset of paralysis. Similar evidence was afforded by eleven cases of Lucas and by one of Frissell.[48] In none of these twelve cases,

[48] Frissell, *Jour. Am. Med. Assn.*, 1911, lvi, 661.

however, was a large percentage of polynuclear cells found. But it seems of importance to call attention to the fact that a fluid whose cytology resembles that found in cerebrospinal meningitis and other types of purulent meningitis may also occur in the early stages of poliomyelitis.

The question arises as to whether there is any relation between the situation and extent of the paralysis or the severity of the disease and the character of the spinal fluid. Conclusions on this point cannot be drawn from the number of cases seen by us. It would seem, however, that in cases in which the paralysis is limited to arms or to cranial nerves, the lumbar puncture fluid is rather apt to show less deviation from the normal than where the legs are involved. This is, however, far from being a constant finding. Several cases which had acute symptoms (pain, irritability) lasting for an unusually prolonged period, gave an unusually large and persistent globulin reaction. Moreover, the disappearance of the acute symptoms was frequently coincident with a diminution in the globulin in the spinal fluid. The fatal cases showed nothing in the fluid on which to base an unfavorable prognosis.

Since the infecting organism in poliomyelitis is too small to admit of its being seen, even if it is present in the cerebrospinal fluid, and since the more complicated biological tests have failed to prove the presence of antibodies in the fluid, the diagnostic value of examinations of the spinal fluid by simple chemical and microscopic methods must necessarily be merely relative. While in cerebrospinal meningitis, in tuberculous meningitis, in pneumococcus, influenza, and similar types of meningitis, one obtains specific information and is usually enabled to make a positive diagnosis from an examination of the spinal fluid, the value of the examination in poliomyelitis is necessarily less direct. Thus, in a fluid containing a high percentage of polymorphonuclear cells, the failure to find any organisms would certainly be suggestive of poliomyelitis. The difficulty of differential diagnosis is perhaps greater when one has a fluid with a lymphocytic cytology. Lucas has discussed at length the non-specificity of the cytological findings in various meningeal conditions, and finds a similar cell picture in encephalitis, poliomyelitis, meningism, tuberculous meningitis, and syphilis of the

central nervous system. Because, however, the examination of the spinal fluid in poliomyelitis lacks specific diagnostic value, it would be wrong to conclude that it was not an important aid in diagnosis, or that lumbar puncture should not be performed in suspicious cases. Routine blood examinations and the usual urinary analyses have comparatively rarely a specific diagnostic value. Their greatest usefulness consists in helping to rule out certain possibilities, and in focusing the attention on a narrower group. Within such a group the blood or urine examination, partly by itself, and partly when considered with clinical experience, may, by bringing either negative or positive evidence, be the determining factor which points towards the correct diagnosis. The value of positive findings in spinal fluid examinations is undoubted. The value of negative results in the examination approaches it in importance. Thus in the case of poliomyelitis, the failure to demonstrate specific changes in the spinal fluid should rule out several serious meningeal conditions, the differential diagnosis of which from poliomyelitis in the preparalytic stage may be most confusing. On the other hand, by the demonstration of non-specific changes,—for we have seen that by far the greater number of fluids in poliomyelitis are in one way or another abnormal,—a host of other conditions which may simulate incipient poliomyelitis are removed from further consideration. Among the small group of remaining possibilities, certain characteristic types of fluid, as, for instance, one with a high cell count and a normal globulin reaction, may be of some value in deciding the diagnosis. More careful studies of the fluids in conditions other than poliomyelitis may throw further light on the differential value of non-specific fluids. At any rate, when even the non-specific fluid of poliomyelitis is considered in association with the clinical features of the case, the diagnosis can probably be made in the great majority of cases even in the preparalytic stage. The ease and safety with which lumbar puncture can be performed and the simplicity of the examination of spinal fluids should make the method much more widely used than it is at present. The value of any future method of treatment of poliomyelitis must depend on the possibility of early diagnosis, for where the nerve cells have been destroyed, the results from any therapeutic measures will be comparatively small.

Conclusions.—The spinal fluid from cases of acute poliomyelitis during the first few weeks after the onset of symptoms shows, in the great majority of instances, deviations from the normal.

Fluids taken during the early days of the disease and especially before the onset of paralysis tend to show an increased cell count with a low or normal globulin content. At this early stage the polymorphonuclear cells may amount to 90 per cent. of the total. Most fluids, however, show almost exclusively lymphocytes and large mononuclear cells.

After the first two weeks the cell count usually drops to normal, or nearly normal, and there is frequently an increase in the globulin content. A slight increase in globulin may persist for seven weeks or longer.

Analogous changes may be found in the spinal fluid of abortive cases.

All fluids examined reduced Fehling's solution.

The examination of the cerebrospinal fluid in acute poliomyelitis, while giving, as far as is yet known, no specific diagnostic criteria, is of the utmost value as an aid to diagnosis both in preparalytic and in abortive cases (see tables, pages 109, 110, 111, and 112).

PROGNOSIS.

Prognosis in poliomyelitis is a complicated problem. If death and recovery were the only results to be foretold, the matter would be no more difficult than in other infectious diseases, but there is the added uncertainty of the paralyses. In a given case seen in the preparalytic stage, for example, the question at once arises as to whether or not paralysis will appear at all. In another case seen on the first or second day, when there are marked general symptoms and when paralysis of a leg or an arm already exists, it is important to decide whether the process in the spinal cord will probably advance or not. Finally the question of residual paralysis must be considered.

The literature of poliomyelitis is fairly rich in mortality statistics, which form, perhaps, the best basis for prognosis as to life or death. Wickman's series of 868 cases showed a death rate of 16.7 per cent. The figures of numerous other observers are approxi-

TABLE OF EXAMINATIONS OF CEREBROSPINAL FLUIDS.

The numerator of the fraction signifies the day of disease; the denominator is the day of the paralysis. The figure following is the number of cells per c.mm. of spinal fluid. o = normal globulin test; v.s. + = faint precipitate (above normal); s. + = well marked cloud or slightly flocculent precipitate; + = precipitate of large flocculi; and + + = heavy flocculent precipitate with the butyric acid test.

Number of case.	1st week.	2d week.	3d week.	4th week.	5th week.	6th–10th week.
1	$\frac{6}{2}$ 31 v.s. +	$\frac{12}{8}$ 7 v.s. +	$\frac{17}{18}$ 6 v.s. +			
2	$\frac{2}{0}$ 880 + $\frac{3}{1}$ 627 $\frac{4}{2}$ 1,221 +					
3	$\frac{6}{8}$ 7 + +	$\frac{12}{12}$ 5 +				
4	$\frac{7}{3}$ 10 s. +	$\frac{11}{7}$ 46 s. +	$\frac{20}{15}$ 5 s. +	$\frac{27}{22}$ 4 v.s. +		
5					$\frac{34}{32}$ 6 s. +	
6		$\frac{15}{12}$ 4 + + $\frac{18}{15}$ 4 + + +		$\frac{22}{19}$ 6 s. + $\frac{28}{25}$ 7 s. +		$\frac{37}{24}$ 2 s. +
7		$\frac{8}{6}$ 13 + + $\frac{12}{10}$ 19 s. +	$\frac{16}{14}$ 7 s. +	$\frac{24}{23}$ 6 v.s. +		
8	$\frac{3}{2}$ 37 s. +	$\frac{9}{8}$ 12 s. +	$\frac{16}{15}$ 9 o			
9						$\frac{46}{45}$ 30 + $\frac{54}{53}$ 23 s. +
10			$\frac{16}{14}$ 7 s. +	$\frac{23}{21}$ 3 v.s. +		
11	$\frac{3}{1}$ 120 o					
12				$\frac{25}{21}$ 1 o		
13	$\frac{6}{5}$ 30 s. +					
14		$\frac{10}{3}$ 21 s. +				
15	$\frac{5}{?}$ 60 o $\frac{6}{?}$ 64 s. +					
16	$\frac{5}{4}$ 39 s. +	$\frac{13}{12}$ 13 +		$\frac{22}{21}$ 7 v.s. +		
17			$\frac{18}{25}$ 29 +			$\frac{38}{36}$ 3 s. +
18			$\frac{19}{12}$ 16 + + +	$\frac{27}{20}$ 20 + + +		$\frac{39}{32}$ 16 +
19					$\frac{35}{32}$ 165 s. +	$\frac{41}{38}$ 979 s. + $\frac{43}{40}$ 154 s. + $\frac{47}{46}$ 31 v.s. + $\frac{55}{52}$ 5 o

TABLE OF EXAMINATIONS OF CEREBROSPINAL FLUIDS (CONTINUED).

Number of Case	1st week.	2d week.	3d week.	4th week.	5th week.	6th–10th week.
20		$\frac{4}{3}$ 36 + $\frac{14}{10}$ 154 s.+			$\frac{14}{27}$ 12 s.+	$\frac{37}{42}$ 20 + 5 0
21		$\frac{10}{9}$ 10 + $\frac{1}{13}$ 6 +	$\frac{10}{19}$ 6 +	$\frac{27}{26}$ 2 s.+	$\frac{34}{33}$ 11 s.+	
22	$\frac{5}{3}$ 174 0	$\frac{8}{6}$ 44 0 $\frac{10}{8}$ 119 s.+				
23		$\frac{12}{11}$ 11 +	$\frac{20}{19}$ 12+			
24	$\frac{2}{0}$ 6 0 $\frac{3}{0}$ 62 s.+ $\frac{8}{0}$ 59 s.+	$\frac{11}{0}$ 5 0				
25		$\frac{9}{9}$ 12 v.s.+	$\frac{16}{16}$ 8 v.s.+	$\frac{23}{23}$ 10 v.s.+	$\frac{34}{34}$ 4 0	$\frac{41}{41}$ 4 0
26	$\frac{5}{5}$ 16 s.+ $\frac{7}{7}$ 10 v.s.+	$\frac{14}{14}$ 10 0				
27		$\frac{11}{11}$ 1 ++ $\frac{14}{10}$ 6 +	$\frac{21}{18}$ 2 s.+	$\frac{28}{25}$ 2 v.s.+		
28			$\frac{16}{4}$ 6 ++	$\frac{22}{8}$ 11 s.+ $\frac{28}{14}$ 4 0	$\frac{35}{21}$ 10 0	$\frac{44}{30}$ 4 0
29	$\frac{5}{3}$ 10 s.+	$\frac{8}{6}$ 14 ++ $\frac{13}{12}$ 3 ++	$\frac{19}{17}$ 2 ++	$\frac{27}{25}$ 5 ++	$\frac{34}{32}$ 5 +	$\frac{43}{41}$ 2 + $\frac{50}{48}$ 3 v.s.+
30	$\frac{4}{4}$ 55 v.s.+ $\frac{7}{7}$ 295 s.+					
31	$\frac{4}{0}$ 650 +					
32	$\frac{4}{1}$ 61 0 $\frac{6}{3}$ 147 v.s.+	$\frac{12}{9}$ 7 +	$\frac{19}{16}$ 1 s.+			
33	$\frac{4}{1}$ 160 +					
34	$\frac{7}{5}$ 58 s.+	$\frac{13}{11}$ 23 s.+	$\frac{21}{19}$ 18 s.+			
35	$\frac{5}{3}$ 297 +	$\frac{14}{12}$ 38 ++	$\frac{19}{17}$ 27 ++	$\frac{26}{24}$ 8 +		$\frac{38}{36}$ 13 s.+
36		$\frac{12}{7}$ 26 s.+	$\frac{16}{11}$ 12 +	$\frac{23}{18}$ 7 s.+	$\frac{30}{25}$ 4 v.s.+	
37	$\frac{5}{2}$ 69 s.+	$\frac{13}{10}$ 9 s.+		$\frac{27}{24}$ 8 v.s.+	$\frac{34}{31}$ 3 0	
38			$\frac{15}{12}$ 12 s.+ $\frac{21}{18}$ 11 s.+	$\frac{28}{25}$ 14 0		
39		$\frac{12}{11}$ 8 v.s.+	$\frac{17}{16}$ 3 +	$\frac{23}{22}$ 4 v.s.+	$\frac{30}{29}$ 2 v.s.+	
40		$\frac{8}{6}$ 13 s.+ $\frac{13}{11}$ 18 +	$\frac{17}{14}$ 7 +	$\frac{22}{19}$ 24 s.+	$\frac{29}{26}$ 3 s.+	

TABLE OF EXAMINATIONS OF CEREBROSPINAL FLUIDS (CONTINUED).

Number of case.	1st week.	2d week.	3d week.	4th week.	5th week.	6th–10th week.
41	$\frac{7}{?}$ 22 0					
42			$\frac{17}{2}$ 4 0	$\frac{27}{?}$ 9 +	$\frac{29}{?}$ 3 v.s.+	
43	$\frac{7}{6}$ 12 v.s.+	$\frac{12}{11}$ 3 +	$\frac{19}{18}$ 11 s.+	$\frac{26}{25}$ 155 +	$\frac{30}{29}$ 29 s.+ $\frac{34}{33}$ 49 +	$\frac{47}{45}$ 10 0
44	$\frac{7}{5}$ 39 s.+	$\frac{12}{10}$ 29 +	$\frac{17}{15}$ 53 v.s.+		$\frac{33}{31}$ 12 0	
45	$\frac{6}{4}$ 33 v.s.+		$\frac{15}{13}$ 1 s.+	$\frac{22}{20}$ 3 0		
46	$\frac{6}{0}$ 37 0	$\frac{8}{2}$ 44 v.s.+ 10 57 0	$\frac{16}{10}$ 11 v.s.+	$\frac{22}{16}$ 12 v.s.+		
47	$\frac{7}{3}$ 15 +	$\frac{12}{8}$ 12 +	$\frac{19}{15}$ 25 +		$\frac{30}{26}$ 2 v.s.+	
48	$\frac{5}{3}$ 55 0	$\frac{9}{13}$ 15 + 22 +	$\frac{18}{16}$ 2 +	$\frac{27}{25}$ 3 +	$\frac{34}{32}$ 2 s.+	$\frac{39}{38}$ 4 s.+ $\frac{47}{45}$ 3 v.s.+
49		$\frac{9}{4}$ 8 v.s.+	$\frac{16}{11}$ 4 v.s.+	$\frac{23}{18}$ 4 0		
50	$\frac{6}{4}$ 42 s.+	$\frac{10}{8}$ 6 + $\frac{14}{12}$ 30 +		$\frac{23}{21}$ 7 + •		$\frac{36}{34}$ 4 s.+ $\frac{50}{48}$ 6 s.+
51		$\frac{8}{4}$ 83 s.+ $\frac{13}{9}$ 23 v.s.+	$\frac{17}{13}$ 11 s.+			
52	$\frac{5}{4}$ 58 s.+	$\frac{9}{8}$ 5 s.+	$\frac{17}{16}$ 6 s.+	$\frac{24}{23}$ 3 +	$\frac{31}{30}$ 4 s.+	$\frac{47}{46}$ 1 v.s.+
53	$\frac{2}{0}$ 113 0 $\frac{4}{2}$ 239 v.s.+ $\frac{6}{4}$ 112 v.s.+	$\frac{13}{11}$ 5 s.+	$\frac{15}{13}$ 36 v.s.+	$\frac{23}{20}$ 12 +		$\frac{38}{35}$ 4 v.s.+
54	$\frac{3}{2}$ 133 0		$\frac{15}{14}$ 18 +	$\frac{22}{21}$ 4 s.+		$\frac{40}{39}$ 3 0
55	$\frac{4}{2}$ 20 v.s.+	$\frac{8}{6}$ 55 v.s.+	$\frac{15}{13}$ 10 +			
56		$\frac{8}{6}$ 40 + $\frac{12}{10}$ 35 v.s.+	$\frac{17}{15}$ 9 +		$\frac{31}{29}$ 8 s.+	$\frac{47}{45}$ 6 v.s.+
57	$\frac{3}{2}$ 196 0	$\frac{12}{11}$ 8 s.+	$\frac{20}{19}$ 5 s.+			
58	$\frac{2}{0}$ 94 0					
59	$\frac{6}{3}$ 12 v.s.+	$\frac{11}{8}$ 8 s.+		$\frac{23}{20}$ 8 s.+		
60	$\frac{4}{2}$ 18 0	$\frac{8}{6}$ 90 v.s.+	$\frac{16}{13}$ 3 s.+	$\frac{27}{24}$ 3 0		
61	$\frac{4}{2}$ 203 0	$\frac{12}{10}$ 18 +	$\frac{16}{14}$ 6 +			
62					$\frac{32}{26}$ 2 s.+	
63	$\frac{?}{1}$ 473 s.+					

TABLE OF EXAMINATIONS OF CEREBROSPINAL FLUIDS (CONTINUED).

Number of case.	1st week.	2d week.	3d week.	4th week.	5th week.	6th–10th week.
64	$\frac{3}{2}$ 61 0 $\frac{5}{1}$ 320 v.s. +	$\frac{2}{2}$ 60 0 $\frac{11}{10}$ 25 0	$\frac{12}{11}$ 11 v.s. +			
65	$\frac{9}{1}$ 52 s. +	$\frac{14}{11}$ 14 s. +			$\frac{32}{25}$ 7 v.s. +	
66	$\frac{9}{1}$ 22 v.s. +	$\frac{8}{6}$ 114 v.s. +				
67					$\frac{61}{52}$ 4 0	
68	$\frac{6}{2}$ 26 0	$\frac{10}{6}$ 5 +	$\frac{12}{12}$ 5 v.s. +			
69	$\frac{4}{33}$ 90 0	$\frac{10}{9}$ 14 0	$\frac{20}{19}$ 5 0			

mately the same, the usual extremes being 10.8 per cent., reported by Zappert from the epidemic in Northern Austria of 1908, and 22.5 per cent. reported by Lindner and Mally[49] from the epidemic in East Austria in the same year. The mortality, furthermore, varies somewhat with the age of the patients. In younger children the outlook is better than in older ones and adults. Thus, Wickman found the death rate per cent. in patients below eleven years of age to be 11.9, and in cases between the ages of twelve and thirty-two, 27.6 per cent. Our series of seventy-one hospital cases showed a mortality of about 14 per cent. The figure, however, becomes much lower if the total number of cases applying for admission be used. In this total the death rate is 6.8 per cent. The hospital cases represent, in general, the sickest patients that applied.

While such statistics are of great value in giving a general conception of the fatality of the disease, they are of little help in the presence of a given case of poliomyelitis. This, of course, is true in some measure of all fatal diseases, but with acute poliomyelitis there is a peculiar element of chance not present in other general infections; namely, the accident of the lesion destroying simultaneously the phrenic and intercostal centers, an accident which is invariably fatal. Unfortunately, there is no way of knowing where the lesion will occur, or if an existing lesion will advance. Flaccid legs, arms, or facial muscles point only to the cord segment most seriously injured when the case is first observed, and form no cri-

[49] Lindner and Mally, *Deutsch. Ztschr. f. Nervenh.*, 1910, xxxviii, 343.

terion of the extent or subsequent behavior of the lesion. Anterior horn cells that lie immediately outside the zone involved by the pathological process may continue to functionate properly, so that the proximity of the lesion to the phrenic and intercostal centers does not necessarily make the outlook worse. This and the fact that in most cases the initial lesion in the cord is the final one, and rarely advances, is perhaps the most encouraging knowledge that we command in attempting to make a prognosis as to life; for, as was pointed out in the section on paralysis, death in uncomplicated poliomyelitis invariably results from failure of the muscles of respiration. In other infectious diseases where death has been considered to depend upon toxemia, mortality statistics represent more nearly an average failure of human resistance, affected less, perhaps, by the element of chance. Consequently, at the bedside of a patient acutely ill with poliomyelitis, where the outward signs of the state of the disease are, from the nature of the malady, so unsatisfactory, the physician experiences an unpleasant sense of obscured vision. There are certain clinical features, however, which may help us in some measure to form an idea upon which to base prognosis as to life. Death, according to Wickman, occurs most often on the fourth day of the paralysis, the third to the seventh being the limit. In our cases, death occurred once on the second, four times on the third, once on the fourth, and once on the fifth day of the paralysis. Figuring, however, not in days of paralysis, but rather from the onset of the disease, the fifth day has been the most fatal, with limits from the fourth to the eighth days of the disease. We therefore made it a rule not to declare children out of danger until after the eighth day from the first appearance of muscular weakness.

Usually, the fatal cases are very ill in the first two or three days, and in our series all had paralysis of one or both deltoids; that is, an involvement of the cervical cord. The extreme prostration and the upper extremity paralysis, unless the case was of the rapidly ascending type (Landry), have been the only tangible prognostic features. An impression, however, derived largely from the patient's psychic state, has helped materially. In practically all the fatal cases the peculiarly alert cerebration, described under the sec-

tion on the fatal cases, has been present. None of the profoundly stuporous or highly irritable cases have died. Consequently, we have been glad to see patients in the early days either irritable or drowsy. One case, in particular, recovered, whose rapidly ascending paralysis, first involving both legs, then one arm, then the back and neck, had led us to give a bad prognosis. This patient was very drowsy, and was irritable if disturbed.

Paralysis of either diaphragm or thoracic musculature alone is not necessarily of bad prognostic significance. Children with such involvement, however, are rather prone to develop bronchopneumonia, which is then almost always fatal. Nevertheless, we saw one instance in a boy of twenty-one months, who reached the hospital with a paralyzed diaphragm and a resolving consolidation of lobar pneumonia. He made a good recovery.

Another equally difficult question in the prognosis in poliomyelitis is that which arises during the preparalytic stage; namely, will paralysis occur, or not? As in the rapidly advancing cases, where it is impossible to know whether or not the respiratory muscles are about to fail, so here the same sense of obscured vision baffles the physician. We have found absolutely no certain way of anticipating paralysis. Occasionally the patient may complain of pain in a member which is subsequently lamed. This is unsatisfactory, however, because pain in general is such a common feature of the disease. Furthermore, the disappearance or absence of a knee jerk is no infallible signal of approaching weakness. One abortive case which we observed practically from the day of onset throughout the course, was the source of hourly apprehension for eight days. At one time, on the third day of the disease, the knee jerks, which had been exaggerated, became much less easy to obtain. Three days later the left knee jerk reacted on reënforcement only, but no paralysis developed. In a similar way it is impossible to determine whether or not an advancing paralysis is about to stop. As a rule, although figures on this point are inaccurate and unconvincing, our experience has been that the initial paralysis is final. Nevertheless, there are enough examples of late involvements to make the anticipation of further paralyses quite justified at any time until the seventh or eighth day.

A discussion of the prognosis in poliomyelitis necessarily involves a consideration of the ultimate disability caused by the disease. It was formerly supposed that the paralysis was always permanent. Wickman's extensive studies, however, and the report of the Massachusetts State Board of Inquiry show that this fortunately is far from the truth. For example, of the 530 cases, one to one and a half years after the acute attack, which were analyzed by Wickman, 56 per cent. were paralyzed, and 44 per cent. were cured. The records from Massachusetts indicate a considerably lower percentage of complete recoveries,—16.7 per cent. Complete return of power is more apt to occur in children than in adults. It is still too early to be able to report figures of value from our hospital cases. We have, however, been much struck by the surprising return of power which may occur after a few weeks in limbs that seemed hopelessly paralyzed, and we have been even more impressed with the marked improvements occurring after several months of a stationary condition of the paralyses.

TREATMENT.

At the present time there is no specific form of therapy by which the paralyses in acute poliomyelitis may be prevented, or by means of which resolution of the inflammatory process and, consequently, return of function may be hastened. The problem of treatment, therefore, consists in preventing the spread of the disease to other persons, in applying general symptomatic procedures, and in attempting the restoration of muscular efficiency and the prevention of deformities.

Inasmuch as the direct contagiousness of poliomyelitis and its dissemination by healthy intermediaries are now definitely established facts, the maintenance of strict quarantine is essential for the public health. In this disease, which in its sporadic form has been with us so long, and towards which there is apparently a high individual immunity, it is peculiarly difficult to convince many persons, both lay and medical, that a quarantine is not somewhat superfluous. Recent investigations, however, which show that in some epidemics the number of families or houses with more than one case may reach 40 per cent.; that "persons have been attacked by poliomyelitis

several days after a short and single contact with a patient";[50] that markets, fairs, schools, and public gatherings may be the means of spreading the disease; that transmission by fomites probably occurs; that the virus may exist in the saliva and nasal mucous membrane in monkeys and in the tonsils in man; and that it may also be found in the dust from the rooms of patients, have convinced public health authorities that poliomyelitis should be treated like any other definitely contagious disease. In Sweden, Norway, Germany, many provinces of Austria, and in a number of states in this country, poliomyelitis is one of the diseases in which notification, quarantine, and disinfection are required by law. We have already mentioned the outbreak in Nebraska in 1909, which was apparently checked by imposing an absolute quarantine for three months on all members of a patient's family with the exception of the bread winner. The Paris Academy of Medicine recommends the passage of a law which would "allow the interdiction of school attendance for three months, and would apply equally to patients and convalescents." It is probable, however, that the general enforcement of such prolonged quarantine would work hardships which would scarcely be compensated for by the results obtained. The question as to how long the disease ought to be considered contagious is a most difficult one. That the disease is contagious during the prodromal stage and perhaps also during the incubation period is, unfortunately, extremely probable. Some authorities believe that isolation should be continued through the acute stage of the disease, until the subsidence of pain, hyperesthesia, and all acute symptoms. In our own work we have drawn a rather arbitrary line based on the fact that in the experimental disease in monkeys, the virus rarely persists after three or four weeks. That it may persist longer in monkeys, and probably also in human beings, is of course certain, but we have felt that this gave the best practical rule for hospital purposes. We have endeavored, therefore, to isolate the children in the hospital until about four weeks after the onset of the disease. We have then allowed them to go home, but have urged that, when possible, they be kept apart from other small children for several weeks more.

[50] Notification of Poliomyelitis—Translation of a Report Presented to the Paris Academy of Medicine on "Obligatory Notification of Poliomyelitis (Infantile Paralysis)," Public Health Reports, 1911, xxvi, 1602.

During their stay in the hospital, the patients with poliomyelitis were kept in an isolation ward apart from all other patients. Suspected cases with uncertain diagnosis, or cases proving not to be poliomyelitis were placed in separate rooms and completely isolated. Nurses on duty in the ward never came in contact with patients in other parts of the hospital, and the same was usually true of doctors. All doctors and nurses wore caps and long gowns when working with the patients. When leaving the ward, the hands were thoroughly scrubbed with soap and a nail brush, and soaked in corrosive sublimate. The occasional use of a hydrogen peroxide gargle or nasal spray (1 per cent.) was recommended. Visitors were admitted on two days in the week, but only one visitor to a patient. All visitors were compelled to wear gowns while in the ward, to wash carefully and disinfect the hands in corrosive sublimate, and use a hydrogen peroxide mouth wash on leaving the ward. The clothes worn by patients on admission were disinfected by steam under pressure. Bedclothes and nightgowns were passed through a sterilizing washer. Thermometers were kept in corrosive sublimate. Bedpans and urinals were boiled after using. All urine and feces were sterilized by heat in a steam sterilizing hopper. After being used, the rooms were disinfected with formaldehyde gas, and the walls and floors were thoroughly scrubbed with soap and water.

The general treatment of the acute stage of the disease as regards diet, bathing, catharsis, etc., should be carried on exactly as in other infections. As soon as the children showed a desire for a liberal diet, it has been given to them. After they have passed the early acute stage, they are apt to have unusually large appetites. During the acute stage absolute rest in bed is, of course, a necessity in most cases, but even when the children feel well, it is probably wise to keep them quiet for a week or two after the onset of paralysis. At this early period, the most prominent indication for treatment is usually pain. In some instances, as we have seen, pain may be spontaneous, but much more frequently it is associated with movement of the limbs, and this necessitates the utmost gentleness and care in moving or turning the child and changing the bed linen. When lifting or moving a patient with marked hyperesthesia, the nurse should try to avoid flexing the neck or the hips, or bend-

ing the spine forward, as these are the most painful motions. Not infrequently, this pain is so great that the children develop a dread of being touched, and they cry out long before they are actually hurt. The nurse who realizes that their pain may be intensely severe, whose touch is gentle, and whose voice is reassuring, may do much towards preventing this unhappy state of mind. In patients with spontaneous pain or with tender muscles, the weight of the bedclothes may be sufficient to cause discomfort, and a cradle to raise them off the limbs is often a relief. In other cases a light, well padded splint seems to steady the limb and to take the strain from unaffected muscles. One of our children used to beg to have the splint replaced when it was taken off. In general, the most effective simple agent for overcoming pain seems to be heat. This is especially the case in paralyzed limbs which are often very cold and clammy. Wrapping the limb in cotton wool, blankets, hot packs, and hot water bags gives great comfort. We have frequently had occasion to notice the effect of cold weather and dampness on these patients, when a sudden change from warm summer weather to a cold rainy day would be accompanied by definite exacerbations of pain. The ward was, therefore, kept at a warm, equable temperature. A certain proportion of cases suffer so intensely that simple measures are not sufficient to control the pain, and drugs have to be resorted to. Bromides we have found helpful in little children. In older patients phenacetine, aspirine, codeine, and occasionally hypodermic injections of morphine are necessary. We have never felt convinced that lumbar puncture was of any therapeutic value. In one fatal case life was apparently prolonged for a short time by means of artificial respiration. Landolt[51] reports a case in which artificial respiration was carried on almost constantly for seventy-two hours. During this period there was slight return of power in the toes and fingers, but the patient finally died. Theoretically, it would seem that one might possibly tide a person over by artificial respiration until an absorption of exudate allowed the nerve cells controlling the respiratory muscles to resume their function.

During the early part of the disease the most important indica-

[51] Landolt, *Cor.-Bl. f. schweiz. Aerzte,* 1911, xli, 1144.

tion is for complete rest and quiet. When the acute symptoms pass, however, more active treatment directed towards the prevention of deformities and the restoration of muscular function should be instituted. The exact period at which it is best to begin treatment varies, but in general the pain seems to be a good guide. As soon after the first fortnight as the child can bear any movement or handling without pain, massage should be begun. It is important, although sometimes difficult, to distinguish between true pain and the fear of pain which may persist until the child's self-confidence is restored.

Contractures begin to develop early in poliomyelitis, and it is essential that they should be guarded against from the onset. The most common early deformity to be dealt with is the toe drop, occurring from the contraction of the flexors of the toes and extensors of the foot in the absence of normal opposing muscles. Such a deformity is hastened by the pressure of the bedclothes, so that a cradle is useful where it is feared. Sand bags may be used to support a limb; or light apparatus, such as a posterior wire splint with a foot-piece at right angles, may be sufficient to overcome the pull of the muscles. Such apparatus must be well padded and the straps applied loosely so that there is no obstruction to the circulation. The danger of apparatus is that it will keep the limb too quiet and prevent any attempt to use weakened muscles at precisely the period in which both active and passive motions are most to be encouraged. To obviate this, it has been our custom to have the apparatus applied at night only, and to leave the limbs free to move during the day time.

Massage is a most helpful aid to treatment. We have begun with light massage as soon as the pain would allow, and given it twice daily. Its chief usefulness probably consists in assisting the circulation by replacing the effect of lost muscular activity. Heat, and especially baking, also exert a good influence on the circulation. With massage, passive motion is used, the rhythmic performance of certain movements stimulating the patient to try to attempt them himself. Of all methods, however, by far the most valuable one is active movement, or muscle training. In attempting voluntarily to use a weakened or paralyzed muscle, the patient can accomplish

more for himself than can ever be done for him by anyone else. Muscle training is comparatively easy to carry out with adults or children old enough to do what they are told, but with little children, who by instinct avoid the use of any injured muscle, and try only to accomplish the same result by means of another healthy group of muscles, the problem is more difficult. In one way or another, the child must be made to attempt to use the weak muscles. It is here that the skill of the nurse shows itself. By merely telling the child to move an arm or leg, she accomplishes nothing, and as likely as not merely makes him suspicious and obstinate; but by playing with him, coaxing him, inventing ingenious games, which involve the use of muscles without his realizing it, and by showing infinite patience and tact, the end is obtained. Frequently, the children use their limbs better when they are in a warm bath than when in bed. The excitement of the tub bath and the fun of playing with floating toys makes them forget their disabilities, and they try new movements much more readily than in bed. Probably also, the heat and the buoyancy of the water make many muscular movements less difficult. Some children are ambitious and from the outset are continually anxious to progress, but with many each new step involves a struggle. One literally has to force them to try to sit up, to try to stand, and then to try to walk. Dr. Charles West[52] discusses some methods of stimulating children to use their paralyzed limbs and then adds, "I need not say that much care and much patience are needed in carrying out any of these suggestions, and not a little of that intuitive love for children which teaches those who are its possessors how to extract fun and merriment from what might in other hands be a most irksome task."

In the therapeutic use of electricity, we have had little personal experience. To be of any value, electrical treatment must be given with considerable skill and over a long period of time. The amount of other work entailed by the large number of patients we have had in the hospital left us no opportunity to take up this method of treatment. The general methods which may be of service, however, are summed up in the following sentences from the report of the

[52] West, Lectures on the Diseases of Infancy and Childhood, 3d edition, London, 1854.

Massachusetts State Board of Health[53] for June, 1909: "In the early stages, galvanism should be used on the nerve trunks and faradism on the muscles, so long as their irritability for contraction is maintained. When the irritability of contraction to the faradic is lost, galvanism should then be used, as having more influence on nutrition. With the returning muscle irritability, faradism should be used, and best by the use of the electrodes over the muscle points so as to obtain actual contraction of muscles rather than by the application of the electrical current to broad surfaces. This serves as a distinct exercise to the muscle during its early stage of weak contraction."

The results of treatment depend to a great extent on the faithfulness with which it is persisted in. Coincident with the absorption of the exudate, improvement is often quite rapid during the first weeks and months; then follows a long period which may be intensely discouraging in its apparent results, a period in which improvement is probably due to muscle training and to the assumption of new functions by the surviving nerve cells. During this period the patient learns many tricks by which surviving muscles may be made to assume in part the rôle formerly played by those which have become paralyzed. A slow recovery often continues for a very long time, and to be sure that the muscles have regained all the power that they are capable of, conscientious treatment ought to be continued for at least a year and a half or two years. We have been greatly impressed with the marked improvement which may come after the end of the first year. The problem as to when the case ceases to be a medical one and should be turned over to the orthopedic surgeon is difficult and much discussed. The danger of a too early resort to surgery and mechanical methods of support is that the patient will come to rely too much on these methods and cease developing his muscles to their utmost. Internists and neurologists thus tend to postpone calling in the orthopedic surgeon until they feel satisfied that they have done all in their power. The danger of this delay, on the other hand, is that deformities may set in which make the corrective problem much more difficult, so that the orthopedic surgeon receives the case at a distinct disadvantage.

[53] Report of the Massachusetts State Board of Health, *loc. cit.*

The best treatment would probably mean a coöperation in which the surgeon would foresee and correct deformities and the internist would protect the interest of the injured muscles.

When one member of a family is taken sick with poliomyelitis, the physician must take what measures he can to prevent the spread of the disease to other members of the family and to the community at large. Strict isolation of the individual case and isolation of those who have been in close contact with it during the fortnight preceding onset, are the most efficient measures. This is particularly important with regard to the prevention of contact with children. Attendance at school, church, or other places of public meeting should be forbidden for at least three weeks. As direct treatment for those exposed, urotropin, which by animal experimentation has been shown to have some slight action in destroying the virus, may be given for about a week. Longer administration is not advisable. Nasal and throat sprays or a gargle of 1 per cent. peroxide of hydrogen or of a 0.5 per cent. menthol solution should be used, but only for a few days, for their prolonged use is irritating and may make the throat and nasal passages more susceptible.

CASE HISTORIES.

CASE I.

Sherman R., age, 17 months. Ridgewood, L. I. Admitted, September 20, 1911. Discharged, November 8, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The child has never been sick before.

Present Illness.—Thursday morning, September 13, seven days ago, the patient was perfectly well. In the evening he vomited some chocolate which he had eaten. There was high fever that night and all the next day. The child was drowsy and wanted to be let alone. He had no convulsions, but is said to have been "twitchy." On Saturday his fever fell. The temperature was normal on Sunday, and the doctor thought the child would be well. He was drowsy and dull, however, all day. On Monday he seemed to have difficulty in swallowing and later in the day the doctor noticed that he could not use his legs. He has been constipated. The child is teething.

Physical Examination.—The patient is a well nourished, drowsy, small boy, who is fairly easily aroused and resents being handled. His head is usually held on one side and slightly retracted. *Eyes.*—Pupils are equal and react normally. Ocular movements are normal. *Ears.*—Normal. *Nose.*—No discharge. *Mouth.*—The tongue is heavily coated. Mucous membrane is clear. Pharynx is reddened;

tonsils are a little enlarged. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are palpable and slightly enlarged. *Neck.*—There is a little tendency to retraction. The neck is so stiff that the body can be lifted by the head. This causes pain. *Thorax.*—Well formed, moves properly in respiration, though not widely. *Lungs.*—Clear. *Heart.*—Regular and not rapid. *Abdomen.*—A little full, but not distended, soft, tympanitic, no tenderness, and no masses. Abdomen moves properly in respiration. Liver and spleen are not felt. Abdominal reflex is very faint; cremasteric present. *Extremities.*—Upper apparently normal. Lower: there is complete flaccid paralysis of the right lower extremity, and complete flaccid paralysis of the left lower extremity, except for a little power of flexion of the toes and extension of the foot. Knee jerks are absent on both sides. Achilles reflex is present (?) on the right, absent on the left. Babinski reflex is absent on both sides. There is no resistance on testing for Kernig's sign and no spasm, but there is pain at the end of the manipulation. *Temperature.*—98.8° F.

September 20. Blood count: leukocytes, 13,400; differential count of 200 cells: polymorphonuclears, 67.5 per cent.; transitionals, 0; lymphocytes, 28 per cent.; basophiles, 0; large mononuclears, 7 per cent.; eosinophiles, 2 per cent.; stimulation form, 0.5 per cent. Spinal fluid: 30 c.c. of clear, limpid fluid; pressure slightly increased (child crying); cells, 15; globulin, +; sugar, +.

October 19. During the past two or three days there has been a striking improvement in the patient's general condition. He is bright and plays. He is also able to sit up alone in bed without the support of pillows. There is no return of power in the paralyzed muscles.

October 25. There is return of power in the flexors of the toes on the left and in the iliopsoas. The adductors are still paralyzed. The general condition is very good.

CASE 2.

William S., age, 2 years. New York City. Admitted, July 14, 1911. Discharged, September 13, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in three rooms on the first floor in the rear. Air and light are good. The baby sleeps with the mother. No other children in the neighborhood are paralyzed.

Past History.—The baby has always been healthy.

Present Illness.—The child was taken sick July 7, one week ago. He seemed to have fever. The mother gave him castor oil and spirits of nitre, and the fever seemed to leave him. The baby went outdoors and played. Two days later he became feverish again. He was put to bed and given warm baths. The mother first noticed weakness in the legs two days ago. This came on in both legs at the same time. He limped on trying to walk, and said his foot hurt. It was first noticed this morning that the baby could not move his legs at all. There has been no nasal discharge, no eruption, no vomiting, and no chill. He has not cried, has slept most of the time, and has been dull. The bowels have been slightly constipated.

Physical Examination.—The child is a mulatto, lies quietly in bed, awake, with head turned to the left and slightly retracted. The skin is moist and warm. *Head.*—The head is well formed, fontanelle closed. Frontal eminences are

prominent. *Eyes.*—No ptosis. Scleræ and conjunctivæ are clear. Movements of the external ocular muscles are good. Pupils are equal, not dilated, react to light. *Ears.*—No discharge. No mastoid tenderness. *Mouth.*—Lips and mucous membranes are of good color. Teeth are well developed and in good condition. The tongue has a moist, white coat. Tonsils and pharynx cannot be seen. *Neck.*—The child keeps his head somewhat retracted and resists extension slightly. Other movements are easy. *Superficial lymph nodes.*—The cervical, axillary, and inguinal glands are all enlarged, easily palpable, somewhat shotty, and freely movable. *Thorax.*—The thorax is fairly well formed, symmetrical, and moves easily with respiration. The costal angle is very wide, with some flaring of the costal margin on both sides. There is some enlargement of the costochondral joints. Breathing is not rapid, rather shallow. Expiration is about equal on both sides. The lungs are everywhere resonant on percussion and are clear on auscultation. The breath sounds are easily heard and are vesicular in character. *Heart.*—Negative. *Abdomen.*—Protuberant and is everywhere soft; there is no resistance or tenderness, no masses, some rigidity in the region of the bladder which seems full. *Liver.*—Dullness extends from the fourth interspace to 2 cm. below the costal margin in the mammary line where the edge is easily palpable. *Spleen.*—Not felt. *Extremities.*—Upper: the movements of both arms seem normal, and there is no apparent weakness, the biceps reflex is easily obtained on both sides. Lower: both legs are perfectly limp and show a complete flaccid paralysis. On stimulating the soles of the foot, although the child cries and tries to draw the foot away, he is not able to do so. The patellar and Achilles reflexes are absent on both sides. Plantar stimulation gives plantar flexion of the great toes on both sides. *Sensorium.*—No areas of hyperesthesia can be made out. *Temperature.*—100.8° F.

July 15. Blood count: leukocytes, 20,000; differential count of 200 cells: polymorphonuclears, 57 per cent.; eosinophiles, 6.5 per cent.; small mononuclears, 23.5 per cent.; transitionals, 4.5 per cent.; large mononuclears, 8.5 per cent.; mast cells, 0; red cells, normal in size and shape, no nucleated forms seen.

July 16. Spinal fluid: 20 c.c. of clear, colorless fluid; pressure not especially increased; cell count, 36 per c.mm.; stained smear, mononuclears, 98 per cent.; polynuclears, 2 per cent.; globulin, +; albumin, +; sugar, +.

The child lies with great retraction of the neck and when turned on his back assumes a position of lordosis. He complains of much pain when handled, especially in the legs. No sign of involvement of the neck muscles, arms, back, or abdomen.

July 19. The neck still resists flexion. Von Pirquet reaction is negative.

July 30. The neck is still resistant, but less so. There is no retraction.

August 4. Back and neck muscles are weak; child cannot sit up well. The neck still resists flexion, there is no retraction. Abdominal muscles: on sitting there is bulging of the sides of the abdomen, as if the recti had their normal tone, and the other abdominal muscles were weak. Blood count: leukocytes, 18,100; differential count of 200 cells: polymorphonuclears, 58 per cent.; transitionals, 11.5 per cent.; lymphocytes, 21.5 per cent.; basophiles, 0; large mononuclears, 5.5 per cent.; eosinophiles, 3.5 per cent. Spinal fluid: 15 c.c. of perfectly clear, watery fluid; no blood; pressure not increased; cell count, 20 per c.mm.; globulin, ± (slight flocculent precipitate); sugar, +; smear, all small mononuclears; a few red cells.

September 13. There is very slight power of contraction in the left iliopsoas, also in the flexors of the toes; otherwise there is complete flaccid paralysis of the left leg. There is complete flaccid paralysis of the entire lower right extremity, the knee jerk is absent. The patient can sit up well and is very strong in the trunk and arms. The abdomen is large and prominent. The recti seem to be stronger than the lateral muscles. There is no more pain on spinal flexion. The patient's general condition is good. He is fat and his flesh is firm.

CASE 3.

Leo S., age, 4½ years. Port Richmond, S. I. Admitted, July 15, 1911. Discharged, August 22, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a tenement with four other families. The house is clean. The child has one cousin who had infantile paralysis eight years ago. This girl has seen the child once since it became sick, but previously had not seen the child for a long time and very rarely came in contact with it. There is no history of any other possible contact.

Past History.—Three years ago he had bloody dysentery followed by dropsy and swelling of the feet which lasted two weeks. He had whooping cough as an infant and measles at two years.

Present Illness.—Three weeks ago the child was taken with a headache and fever. The next day he fell on the floor and hurt his heel. Since then he has not been able to stand on his heel, but only on his toes. Two days later he "took a sleepy spell" and would not eat. That night both legs gave way when he was trying to stand. The next morning the doctor saw him and made a diagnosis of sprained ankle. The mother noticed that both legs were powerless. Since then he has been in bed. Two days after the onset of paralysis, he began to complain of pain in the legs. He has not been especially restless, he sweats a good deal after the onset. He has not vomited and has had no chill. On July 1 he was unable to pass urine and on July 2 was taken to the Smith Infirmary. While in the hospital he had to be catheterized for two or three days. Since July 2 he has been at the Smith Infirmary. The mother never noticed that the child was cross-eyed. The patient was transferred here from the Smith Infirmary.

Physical Examination.—The patient is a well developed and nourished boy. The skin is clear and warm, lips and conjunctivæ are of good color. *Eyes.*—The eyes move normally. There is an internal squint in the right eye. Pupils react normally. *Ears.*—Normal. There is no tenderness over the mastoids. *Nose.*—No discharge. *Mouth.*—The tongue is clean and protrudes straight. Teeth are in good condition. Tonsils are slightly enlarged but not ragged. *Neck.*—The neck resists flexion but there is no retraction. *Superficial lymph nodes.*—There are very small lymphatic glands felt in the neck, axillæ, and groins. *Chest.*—Symmetrical and well developed. *Heart.*—Normal. *Lungs.*—Negative. *Abdomen.*—Full, soft, symmetrical, and tympanitic, no masses or tenderness, abdominal reflexes are active. *Liver.*—Flatness extends from the sixth rib to the costal margin. *Spleen.*—Not felt. *Muscular system.*—Arms: muscular movements are normal. Legs: knee jerks, Achilles, Babinski, and Oppenheim reflexes not obtained on either side. Kernig's sign: there is no true limitation of motion, but flexion of the hip with extended leg is very painful. There is no pain on pressure except perhaps over the left calf. On extension of both legs he complains

of pain in the popliteal spaces. *Paralysis.*—There is apparently complete flaccid paralysis of both legs. Child is unable to move any of the muscles. He cannot wriggle his toes and cannot flex the thighs on the hips. There is double toe drop. The child is unable to sit up on account of the pain in his legs. There is apparently no weakness in the muscles of the back. *Temperature.*—99.2° F.

July 16. The temperature is normal, chest clear, and general condition very good. There is a slight internal squint of the right eye.

July 17. Blood count: leukocytes, 12,000; differential count of 200 cells: polymorphonuclears, 58.5 per cent.; small mononuclears, 27.9 per cent.; large mononuclears, 9 per cent.; eosinophiles, 3.5 per cent.; transitionals, 2 per cent. red cells normal; platelets normal.

Spinal fluid: 25 c.c. obtained; pressure slight; slightly bloody; specimen used for cell count was very opalescent; an almost turbid fluid with only a very slight pink tinge. Cell count, 529 per c.mm.; differential count of 200 cells; polymorphonuclears, 45 per cent.; mononuclears, 55 per cent.; globulin, ++; albumen, +; sugar, +.

July 20. Paralysis is unchanged, and is complete in the legs. There is marked pain in the back of the thigh on moving the right leg, and there is also pain on dorsal extension of both feet with legs extended.

Spinal fluid: 25 c.c. of opalescent fluid of slightly yellowish tinge; slight increase of pressure; no clot formed after 24 hours; cell count, 524; smear; differential count of 200 cells: polymorphonuclears, 60.5 per cent.; mononuclears, 26 per cent.; endothelial, 13.5 per cent.; globulin, +; albumen, + (faint cloud); sugar, +.

July 21. The temperature this evening is 101.8° F. The child has a headache and feels miserably. As soon as he is moved he complains of severe pain. This is localized, at times in the legs, at times in the back, and frequently in the abdomen in the region of the navel. It is difficult to make out the cause of the pain, but it is apparently associated with movements of the legs.

July 22. The temperature was normal this morning but has risen again to 103.4° F. The child is very uncomfortable when moved. The neck is quite stiff. The ears, eyes, and throat are negative. Heart and lungs (front) are negative. The abdomen is negative. Knee jerks are absent. It is difficult to localize the pain. He says it is worse at the inner side of the left knee, just below the inner tuberosity of the tibia, but when he is moved he has great pain in the lower part of the back, and back of the thighs. There is no especial tenderness. Leukocytes, 15,100 (82 per cent. polymorphonuclears—wet count).

July 23. The temperature has come down. The child cried a great deal during the night, partly from pain and partly not. The lungs are clear; the abdomen is soft with no tenderness.

July 24. Leukocytes, 14,800 (85 per cent. polymorphonuclears—wet count). The neck is very stiff and markedly retracted and is painful on flexion. Kernig's sign: there is much pain in the legs on an attempt to elicit the reaction; no spasm of muscles.

July 27. The fever is less, and the pain less. Extension of the legs is still painful, and the legs are somewhat tender; there is no retraction. Flexion is less painful, but complete flexion is distinctly painful and is resisted. Chest and abdomen are clear. There is marked atrophy of the legs.

July 28. Blood count: leukocytes, 6,300; differential count of 200 cells: polymorphonuclears, 62.5 per cent.; small mononuclears, 19 per cent.; large mononuclears, 12 per cent.; mast cells, 1 per cent.; transitionals, 3.5 per cent.; eosinophiles, 2 per cent.

July 30. The child looks much better. The head flexes more easily, but flexion is still painful.

August 9. Spinal fluid: 25 c.c. of perfectly clear watery fluid; pressure slightly increased; cell count 30 per c.mm.; smear: small mononuclears, 82 per cent.; large mononuclears, 18 per cent.; globulin, + (slight flocculent precipitate); sodium chloride, 0.74 per cent.

August 16. Spinal fluid: 20 c.c. of perfectly clear watery fluid; no blood; pressure slightly increased; cell count, 23 per c.mm.; mononuclears, + (occasional polymorphonuclears); globulin, slightly +; sugar, +.

August 18. Blood count: leukocytes, 10,700; differential count of 200 cells: polymorphonuclears, 52.5 per cent.; small mononuclears, 35.5 per. cent.; large mononuclears, 9 per cent.; eosinophiles, 0.5 per cent.; transitionals, 2.5 per cent.

August 20. The child is much better and sits up very well. The chest is clear. There is no pain on flexion of the neck and no pain in the legs. The abdominal and back muscles are normal. He can flex the toes of the left foot, otherwise there is complete paralysis of both legs.

August 22. The general condition is much improved. The child has no pain and sits up well, but cannot stand. There has been practically no return of movement in the legs.

CASE 4.

Hannah T., age, 4 years. New York City. Admitted, October 3, 1911. Discharged, October 29, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Past History.—The patient has always been a strong healthy child.

Present Illness.—On Sunday, October 1, two days ago, the child was perfectly well in the morning. In the afternoon she was "kind of heavy and wanted to go to bed." That evening she vomited, slept poorly, and was restless. On Monday morning, when she got up and tried to walk, she fell. She tried again to walk, but again fell. Later in the day she said she felt well and wanted to get up, but on trying to walk, fell a third time. This morning she vomited. She has had no convulsions, has been constipated, and sweat heavily on Sunday night. She is said to have had fever.

Physical Examination.—The patient is a well nourished little girl who lies quietly on her back and tends to be drowsy. When she is aroused, she becomes greatly excited, frightened, and trembles. She objects to being handled. The breath has a heavy, sweetish odor. *Eyes.*—Pupils are equal, react normally. Ocular motions are normal. There is marked photophobia. *Ears.*—Normal. *Nose.*—There are a few, small, dried scabs on the inner margin of the external nares, no secretion. *Mouth.*—The lips are dry, the tongue heavily coated. Buccal mucous membrane is clear. Pharynx is slightly reddened. Tonsils are not very large. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are palpable. *Neck.*—Anterior muscles seem a little weak. Posterior muscles resist attempt at flexion. There is no retraction. *Thorax.*—Well formed, moves properly in respiration. *Lungs.*—Clear. *Heart.*—A little rapid,

otherwise normal. *Abdomen.*—Soft, not distended, no tenderness or masses. *Liver.*—Edge felt about two fingers' breadth below costal margin. *Spleen.*—Edge just felt below costal margin in anterior axillary line. *Extremities.*—Upper: normal. Lower: there are no paralyses, but there is inability to stand. Both hip and knee joints buckle when weight is put on the lower extremities. There is apparently no definite weakness of the glutei, so that the trouble probably lies in weakness of the quadriceps extensors. Knee jerks and Achilles reflexes are absent on both sides. Babinski reflex is absent on the left and on the right. Kernig manipulation is not resisted and causes very little pain. *Temperature.*—101.2° F.

October 3. Blood count: leukocytes, 17,600; differential count: polymorphonuclears, 44.5 per cent.; lymphocytes, 37.5 per cent.; large mononuclears, 3 per cent.; transitionals, 14.5 per cent.; basophiles, 0; eosinophiles, 0; stimulation form, 0.5 per cent.

Spinal fluid: 20 c.c. of clear limpid fluid; pressure not increased; cells, 196; mononuclears, + +; globulin, slightly hazy; sugar, +.

October 5. The child is very irritable and complains of pain on being moved. The left ankle and foot are especially painful. The right quadriceps is apparently paralyzed, also the right peroneal group. The child lies on her side with marked retraction of the neck. There is great pain on flexion of the legs. Examination is difficult because of great irritability and the rigidity with which the body is held.

In the afternoon, the patient complains bitterly of pain in her feet. Complete paralysis is evident in both quadriceps.

October 6. The patient is lying quietly on her left side with head retracted. She is apparently not suffering from pain at this moment. Flexion of the neck is painful. The legs are not especially tender on pressure, but are very painful when she makes an attempt to move.

October 8. The pain seems to be somewhat less, but at times is so severe that she cries out from it. Both legs are very tender to pressure, apparently more so over the nerves. Movements of the legs are very painful, in fact, her legs have to be left in one position and motion cannot be tested.

October 9. The pain is perhaps a little less, but she cannot bear to have her legs moved.

October 10. There is pain when the muscles are squeezed.

October 12. The child still has much pain. She is very much afraid of being touched, as she has had so much pain, and it is hard to tell just how much is pain, and how much is nervousness. She has suffered so that she does not tell the nurse when she wishes to void, or when her bowels move, apparently dreading being put on the bed pan.

Spinal fluid: 15 c.c. of clear watery fluid, no increased pressure; cell count, 8 per c.mm.; globulin, slightly +; sugar, +.

October 14. The child is much better. For the first time she responds when spoken to and gives her hand. The arms move normally. Flexion of the neck is painful. Movement of the legs is still very painful, so that they cannot be tested for paralyses and reflexes.

October 18. The patient says she has not so much pain in the legs this morning, though she complained of it last night. If they are manipulated, however,

she cries out. The left knee jerk is present; the right, absent. The patient apparently has little power over the lower extremities. Flexion of the neck causes pain and the patient resists actively all attempts to make her sit up. She throws the head far back when this attempt is made.

October 20. Spinal fluid: cells, 5 per c.mm.; mononuclears, +; sugar, +; globulin, slightly +.

October 29. There is surprisingly little improvement in the tenderness of the spine and legs. The slightest movement towards flexion of the thigh causes intense pain, and the patient will not and probably also cannot move the lower extremities. The general condition is better. The patient is less moody.

October 29. The patient's mother insisted on taking her home. When the mother dressed her, the child did not complain of pain as she does when doctors or nurses touch her.

CASE 5.

John O., age, 21 months. New York City. Admitted, July 25, 1911. Discharged, September 23, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a tenement house. There are eleven other families in the house. They have five rooms on the ground floor. There is good light and fair ventilation. The only parasites that have been noted are bedbugs. The father states that these are worse in the children's room. The father is in good financial circumstances, is a fireman and has been working on a fireboat for the past eleven months. In his duties he has not been called into any house where there was infantile paralysis; in fact, he has been on the boat constantly.

Past History.—The child has grown well, and has always been in excellent health.

Present Illness.—Friday evening, July 21, four days ago, the child was slightly feverish and his face flushed. He took his supper as usual. The next morning he was still slightly feverish but took his breakfast. He complained of headache and pain in the stomach but could walk as well as usual. In the afternoon the mother took the child out in a carriage, and she noted that when he got out he did not want to walk but preferred to be carried. If supported, however, he could walk almost as well as usual. Two days ago, the fever was more marked, and the parents noted that there was some weakness of the legs. They noticed that when the physician examined him at that time, in stimulating the plantar surfaces of the feet he could wriggle the toes, although he could not flex the legs. He could, however, sit up in bed. Yesterday he could not sit up in bed, and absolutely no movements of the lower extremities could be obtained. Two days ago the fever was 103° F. Yesterday it was somewhat lower. The child has had but little appetite and has taken only milk and a little broth. Castor oil has been given each day, but an enema has been necessary to move the bowels. When the patient was quiet in bed, he did not seem to suffer much pain, but when moved, seemed to have a great deal of pain in the back and legs. This morning he seemed weaker than at any previous time. His cry to-day is noted to be rather weak. There has been no vomiting, and there have been no convulsions.

Physical Examination.—The child lies on his back with his head rotated

to the right. The skin is warm and moist. The child looks dull. *Head.*—Well formed, fontanelles closed. *Eyes.*—No ptosis, no strabismus. Ocular movements are good. Pupils are equal and react to light. Scleræ and conjunctivæ are clear. *Ears.*—No discharge, no tenderness. *Nose.*—No discharge. *Mouth.*—The lips and mucous membranes are of good color. Teeth are well formed, tongue has a moderate white coat. The mouth is drawn slightly to the right on crying. No exudate on the pharynx or tonsils. The right tonsil is enlarged, the left just visible. *Neck.*—The head is rotated to the right, resists rotation to the left or flexion towards the chest. Flexion causes pain. When the child is sitting up, the head drops back, although it can be brought forward. There is evident weakness of the muscles of the neck. *Superficial lymph nodes.*—The cervical and axillary nodes are palpable and slightly enlarged. *Chest.*—Well formed, costal angle wide. The chest moves with respiration slightly. There is more expansion on the left than on the right. Breathing is partly costal and partly diaphragmatic. *Lungs.*—Negative on auscultation and percussion. *Heart.*—No increase in size on percussion. Apex impulse is neither visible nor palpable. Heart sounds are clear and forceful. No murmurs are heard. *Abdomen.*—Well rounded, moves easily with respiration, not distended, no tenderness, no masses or rigidity. *Liver.*—Dullness extends from the fifth interspace to the costal margin in the right mammary line. Edge is not felt. *Spleen.*—Not felt. *External genitalia.*—Normal. *Extremities.*—Upper: movements of the arms seem normal. Brachial reflex is active on the left, not obtained on the right. There is no wrist drop. Lower: there is complete flaccid paralysis of all the muscles of both legs. No reflexes can be obtained, no Kernig's sign. There is no apparent muscular tenderness. *Temperature.*—100.4° F.

July 26. Spinal fluid: clear, colorless, not opalescent; pressure not increased; cell count, 10 per c.mm.; globulin, slightly +; differential count: mononuclears, 88 per cent.; polymorphonuclears, 8 per cent.; endothelial cells, 4 per cent.

July 27. Blood count: leukocytes, 15,700; differential count of 200 cells: polymorphonuclears, 53.5 per cent.; mast cells, 1 per cent.; small mononuclears, 28.5 per cent.; large mononuclears, 12.5 per cent.; transitionals, 4.5 per cent.; eosinophiles, 0.

July 29. Spinal fluid: 20 c.c., for the most part perfectly clear and watery; pressure apparently slightly increased; cell count, 14; smear contains a few red cells; mononuclears, 70 per cent.; endothelials, 20 per cent.; polymorphonuclears, 10 per cent.; globulin, + +; sugar, +.

August 1. Chest and abdomen are clear. The child's general condition improves very slowly. He does not get much brighter and cries or groans most of the time when not left alone. Physical examination does not show sufficient reason for his poor condition.

August 3. Von Pirquet reaction positive in 24 hours. When sitting up, the child can hold his head up for a short time, then it drops backward. The body is not supported from the hips. It falls forward so that the body and the thighs lie almost parallel, the body completely doubled up.

Spinal fluid: 15 c.c. of perfectly clear watery fluid; no increase of pressure; cell count, 3 per c.mm.; globulin, + + (heavy flocculent precipitate).

August 4. Blood count: leukocytes, 22,000; differential count of 200 cells: polymorphonuclears, 46 per cent.; lymphocytes, 38.5 per cent.; large mono-

nuclears, 0.5 per cent.; transitionals, 11 per cent.; basophiles, 0.5 per cent.; eosinophiles, 3.5 per cent.; stimulation form, 0.

August 5. The patient continues to lie in a listless fashion without taking much notice of his surroundings. There is no change in paralysis. The neck is weak in supporting the head when the patient sits up.

August 9. Spinal fluid: 25 c.c. of clear colorless watery fluid; no increase of pressure; cell count, 2 per c.mm.; smear, almost all small mononuclears; an occasional polymorphonuclear; globulin, + + (fairly heavy flocculent precipitate); sodium chloride, 0.72 per cent.

August 14. The child is still hypersensitive and timid. He begins to cry and sob as soon as any one approaches him and starts to examine him. The ne is resistant and flexion painful. When he sits up, the head drops over backwards. Sitting up is apparently very painful. He flexes and extends his hands and forearms, but when trying to raise the upper arms, he hunches his shoulders. There is paralysis of the shoulder muscles on both sides. Mentally, the child is hard to rouse and takes no interest in anything around him except in his food, which he takes well. He sweats very freely. There is a peculiar disagreeable odor to the skin.

August 25. The child seems much brighter and more interested in things. He plays a little. The nurse says he seems less tender when she handles him. He began to talk more yesterday. The paralysis is not changed.

September 3. The child seems rather bright. The head is held steadily on sitting up, though some weakness is still present in the neck muscles. All reflexes of the legs are still absent, paralysis of both legs being still complete. Movements of the right arm are good. The left forearm is weak. There is wrist-drop and the hand is held in a claw-like attitude.

CASE 6.

Jennie A., age, 20 years. New York City. Admitted, July 18, 1911. Discharged, October 5, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The patient has always been healthy.

Present Illness.—On July 14, four days ago, patient had pain in both legs, principally in the thighs. She rubbed her legs with alcohol, and the pain disappeared. The next morning she got up, cooked the breakfast, but afterwards felt feverish, had a very bad headache and vomited, so that she went to bed. Her legs felt better, however. The next morning, July 16, she had less headache and felt pretty well. She stayed in bed, and at one o'clock took a little nap. When she awoke at two, she tried to get up and found she could not move her right leg. Except for inability to move her legs, she has felt perfectly well. On the afternoon of July 17 she was moved to Saint Luke's Hospital. At this time her neck was slightly stiff and movements were painful. She has had no headache and has not felt especially sleepy. She has not vomited since the onset. Her appetite has been poor. Urination has been normal. She has had no pains in her legs since the onset.

Physical Examination.—The patient is a large, well developed girl. The skin is clear and not hot. There is rather marked brownish pigmentation all over the body, more especially over the lower part of the abdomen and upper thighs.

The face is symmetrical. *Eyes.*—The eyes move normally. Pupillary reactions are normal. *Ears.*—No discharge, no tenderness over the mastoids. *Mouth.*—The tongue protrudes straight and has a slightly gray coat. The teeth are in good condition. The tonsils are somewhat enlarged and ragged, but there is no exudate. Throat is otherwise normal. *Neck.*—The isthmus of the thyroid is palpable. There is no stiffness of the neck. *Superficial lymph nodes.*—The lymphatic glands are not enlarged. *Chest.*—Well formed, symmetrical, both sides move equally with respiration, costal angle is ninety degrees. *Heart.*—Normal. *Lungs.*—Normal on auscultation and percussion. *Abdomen.*—Level, soft, tympanitic, no masses or tenderness. The abdominal wall moves normally with respiration. Abdominal reflexes are present. *Liver.*—Flatness extends from the sixth space to the costal margin. Edge is not felt. *Spleen.*—Just palpable on deep inspiration. *Muscular system.*—Legs: reflexes; Kernig's sign, knee jerks, Achilles, Babinski, and Oppenheim reflexes are absent on both sides. Movements of the legs: right leg, complete flaccid paralysis of all of the leg muscles, including the iliopsoas. Left leg, movements are all possible but rather weak. Patient states that it is more difficult for her to move her leg than it was yesterday. Weakness is especially marked in the quadriceps extensor. Sensation is normal on both sides. There is no pain or tenderness over either leg. Arms: muscular movements are strong on both sides. *Temperature.*—99.8° F.

July 19. Blood count: leukocytes, 13,000; differential count of 200 cells: polymorphonuclears, 59 per cent.; small mononuclears, 17.5 per cent.; large mononuclears, 17 per cent.; transitionals, 4.5 per cent.; eosinophiles, 1.5 per cent.; mast cells, 0.5 per cent. Red cells are normal.

Spinal fluid: 15 c.c. of clear colorless fluid, not opalescent; no increase of pressure; cell count, 297 per c.mm.; smear, small mononuclears, 96 per cent.; large mononuclears, 4 per cent.; globulin, +; albumin, +; sugar, +.

July 22. 8:00 P. M. Leukocytes, 10,400. The pain is extreme. The patient no longer has pain in the back, but has great pain all through the thigh, front and back, extending down into the calf. This is especially marked on the right (paralyzed) side. The leg is tender, and there is a spot of especial tenderness over the anterior crural nerve, just below Poupart's ligament. Heat is comforting but does not relieve the pain. There is no pain in the head or stiffness of the neck. The ears and throat are negative. The heart is negative, lungs clear, abdomen soft, no tenderness.

July 23. The patient was given morphia twice and had a fair night. This morning the pain is starting again. The pain and tenderness in the back are less. Left leg: knee jerk is absent, Achilles reflex active. There is no marked pain, but there is tenderness over the sciatic nerve. Right leg: there is pain in the upper and lower leg like a "tired feeling." There is exquisite tenderness on pressure over the course of the sciatic nerve in the posterior thigh, popliteal space, and calf region. Tenderness is fairly accurately localized over the nerve. There is extreme tenderness over the anterior crural nerve just below Poupart's ligament, over the upper front of the thigh, and over the inner aspect of the thigh. Heart, lungs, and abdomen are negative.

July 28. Spinal fluid: 25 c.c. of perfectly clear fluid, not opalescent; pressure increased; cell count, 38 per c.mm.; smear, mononuclears, 99 per cent.; endothelials, 1 per cent.; globulin, ++ (heavy flocculent precipitate). Towards

evening the patient began to have considerable pain, after a very comfortable day. The pain was present over the left lower quadrant of the abdomen and especially over the left gluteal region and thigh. The upper border of quite a definite area of hyperesthesia is at the level of a line extending from the crest of the ileum to somewhat above the pubes. The lower line of the area of tenderness and hyperesthesia on the thigh is less definite. There is marked pain on pressure over the outer left thigh and over the inner thigh below Poupart's ligament. Palpation of the abdomen is painful over the left lower quadrant. The abdomen moves well with respiration. There is no spasm, no mass.

August 2. Spinal fluid: 15 c.c. of clear fluid; no pressure; no blood; cells, 26 per c.mm.; smear showed about 100 per cent. small lymphocytes; globulin, + + (heavy flocculent precipitate).

August 3. Sensation for touch and temperature is normal.

August 4. Blood count: leukocytes, 18,100; differential count of 200 cells: polymorphonuclears, 67.5 per cent.; lymphocytes, 19.5 per cent.; large mononuclears, 3.5 per cent.; transitionals, 9 per cent.; eosinophiles, 1 per cent.

August 6. There is a general improvement but the patient still has considerable pain and needs codeine and often morphine nearly every day. The pain is all through the back and legs, especially the right, and tenderness is most marked over the nerve trunks. There is no more return of function in the right leg.

August 9. Spinal fluid: 25 c.c. of clear limpid fluid; no increase of pressure; no blood; cell count, 7 per c.mm.; all mononuclears; smear, mononuclears, + +; globulin, + ± (heavy white flocculent precipitate).

October 5. The general condition is excellent. Legs: reflexes on the right are absent. On the left the knee jerk is practically absent and Achilles reflex just obtained. Sensation to touch and pain is normal on both sides. The temperature is somewhat higher on the surface of the left leg than on the right. The right calf measures 28 cm., the left 31 cm. The right thigh measures 47 cm., the left 51 cm. Movements of the left leg are normal. Flexion of the right thigh on the pelvis is possible and patient also has some power of adduction of the thigh. Extension of the lower leg is not possible. She has very little power to flex the toes. She can stand on the right leg, if the knee is held stiff, and can walk a little in this way. The patient is transferred to the New York Orthopedic Hospital to have a brace fitted.

CASE 7.

James C., age, 18 months. Brooklyn, N. Y. Admitted, August 1, 1911. Discharged, August 13, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a four family house in the country. The house is eight years old. It is clean and has good air. There are no animals in the house. The mother states, however, that there are lots of bedbugs, cockroaches, and chicken lice.

Past History.—Contains nothing of importance.

Present Illness.—The child was absolutely well until six days ago when, after a nap, he woke up suddenly, and his mother on trying to make him walk, found that he could not stand. He had been playing before that same morning. He vomited once two days later, the vomitus consisting of "curds." He has been very restless but not drowsy. The mother noticed that he would "shake his arms and legs" three or four times during the night. He has sweat profusely

and has cried out with pain on being touched. His appetite has been poor, and he has had a bad cough since onset. He has also had difficulty in urination and has been very constipated. Four days ago the left arm became very weak, but it improved on being rubbed. The right arm also was weak two days ago and appeared to be painful.

Physical Examination.—The patient is a rather thin, well developed baby, lips and skin pale. The skin is clear except for many small brownish papules the size of pinheads over the neck and shoulders (prickly heat?). The child lies quietly with his eyes closed, dozing, and objects to being touched. Anterior fontanelle not quite closed, frontal bosses prominent. Tache cérébrale is well marked but not of long duration. *Eyes.*—Pupils are equal, regular, and react normally. Ocular movements are normal, no strabismus. *Ears.*—No discharge, no tenderness over mastoids. *Nose.*—No discharge from nares, no excoriation. *Face.*—The face is symmetrical at rest, drawn more to the left than to the right on crying. *Mouth.*—The tongue has a moderate grey coat and protrudes straight. The teeth are in fair condition. Tonsils are not enlarged; pillars slightly reddened, no exudate. *Neck.*—The neck is somewhat stiff and resists flexion. *Superficial lymph nodes.*—Small discrete glands felt in neck, axillæ, and groins. *Chest.*—Well formed, symmetrical. Both sides move equally with respiration. Respiration is chiefly abdominal. Costal angle is ninety degrees. *Heart.*—Normal in size. Action is regular and rapid. Sounds are of good quality, no murmurs. *Lungs.*—Clear throughout on ausculation and percussion. *Abdomen.*—Level, soft, tympanitic, no masses or tenderness, symmetrical, moves normally with respiration. Abdominal reflexes are present. *Liver.*—Flatness extends from sixth rib to the costal margin. Edge is felt 3 cm. below the costal margin. *Spleen.*—Not felt. *Muscular system.*—Legs: all reflexes are absent on both sides. Kernig manipulation is painful on both sides. Movements: there is apparently a complete flaccid paralysis of both legs, including the iliopsoas. The only exception noted is power of flexion of the toes of the right foot and slight power of flexion on the left. Arms: movements are normal, but the right arm is weaker than the left. Neck and back muscles are weak. When the child sits up, the head drops forward, backward, or sideways. The spine is not held straight, but the buckling is not constantly towards one side. The back is not well supported from the hips. The child falls forward.

August 1. Blood count: leukocytes, 12,200; differential count of 200 cells: polymorphonuclears, 47 per cent.; small mononuclears, 40 per cent.; large mononuclears, 10 per cent.; transitionals, 2.5 per cent.; eosinophiles, 0.5 per cent.; mast cells, 0.

Spinal fluid: 15 c.c. taken; clear watery fluid, not opalescent; no increase of pressure; cell count, 7 per c.mm.; smear, small mononuclears, 98 per cent.; large mononuclears, 2 per cent. (no blood); globulin, + + (heavy flocculent precipitate).

August 13. The mother came and wanted to take the baby home. No improvement has been noted in paralysis. He was discharged.

CASE 8.

Julia S., age, 2 years and 3 months. New York City. Admitted, September 29, 1911. Discharged, November 12, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The patient has always been a healthy child.

Present Illness.—On Tuesday, September 26, three days ago, the patient was perfectly well and happy. On Wednesday, September 27, she was feverish and lost her appetite. The mother said that she became very drowsy on that day. She did not vomit or cough. There were no convulsions but occasional twitchings of the legs and arms. The patient was constipated. Very little sweating occurred until after the doctor gave some powders to control the fever. Yesterday the patient complained of pain in the neck. She has gradually become more and more stuporous until today she seems to recognize no one.

Physical Examination.—The patient is a well nourished little girl, who lies apparently in coma vigil. For the most part the eye slits are held wide and the expressionless eyes are drawn usually to the right. She can be aroused by handling, and then she whines and cries in an irritated way. She evidently sees, but does not recognize. When a strong light is thrown in the eyes, she attempts to close the lids but does not succeed completely; a narrow slit remains. The eyeballs are rolled upwards synchronously, so that the pupils are covered by the upper lid. The head is held a little retracted and almost constantly on the right, although free voluntary motion is possible in all directions. Occasionally a slight twitch or tremor passes over one extremity or side. At times she lowers the upper lids and dozes off into deep sleep. The face is a little flushed and the lips tinged faintly with cyanosis. She breaths a little more rapidly than is normal. *Eyes.*—Pupils are equal and react to light. Sometimes a fine nystagmus is seen when the eyes follow an object well to the side. Occasionally slight incoördination of the eyeballs is seen. There is slight exophthalmos. *Ears.*—Normal. *Nose.*—No discharge. *Mouth.*—The tongue is heavily coated, and there is much thick greyish material posteriorly. The mucous membrane is reddened throughout, especially in the pharynx. The tonsils are very large and look almost inflamed. Teeth are in fair condition. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are palpable. The posterior cervical are enlarged. *Neck.*—The neck is markedly stiff to flexion anteriorly, though the impression is gained that much of the resistance is voluntary and not reflex. The thyroid gland seems to be enlarged, especially the isthmus. *Back.*—Patient cannot be made to sit up because of resistance offered to flexing the spine. *Thorax.*—Well formed, moves properly in respiration. *Lungs.*—Clear. *Heart.*—Normal. *Abdomen.*—Soft, no tenderness, no masses. Spleen and liver are not felt. *Extremities.*—No weaknesses are found in either upper or lower. Knee jerks are obtained with difficulty on the right, absent on the left. Babinski reflex is absent. Kernig's sign: there is no resistance to complete extension of the leg on the flexed thigh. No complaint of pain on the right side, very slight objection at end of manipulation on the left. Achilles reflexes are slightly active on the right, absent on the left. *Temperature.*—102.8° F.

September 29. Spinal fluid: clear limpid fluid, pressure very slightly increased; cells, 113 per c.mm.; polymorphonuclears, 30 per cent.; mononuclears, 83 per cent.; globulin, very slightly hazy (normal); sugar, +.

Blood count: leukocytes, 14,200; differential count of 200 cells: polymorphonuclears, 58.5 per cent.; lymphocytes, 31.5 per cent.; large mononuclears, 7 per cent.; transitionals, 3 per cent.; basophiles, 0; eosinophiles, 0; stimulation form, 0.

September 30. General picture this morning is about the same. The child lies in a sort of stupor with eyes partly closed. She cries irritably on being aroused by prodding. There is no facial weakness, but the mask-like expression is striking. There is complete flaccid paralysis of the left deltoid and biceps, and perhaps also of the triceps. The left quadriceps responds very slightly to the patellar tap. This was not noted yesterday, probably on account of incorrect observation. The diaphragm is working. There is possibly a little weakness of the right shoulder muscles. The temperature remains at 102.4° F.

Tuberculin test (cutaneous) applied at 5:20 P. M.

October 1. Von Pirquet reaction negative this morning. The child seems certainly no worse. There is no advance in paralyses.

Spinal fluid: 17 c.c. of very slightly turbid fluid, no increase of pressure; cells, 239 per c.mm.; polymorphonuclears, 4 per cent.; mononuclears, 96 per cent.; globulin, very slightly +; sugar, +.

October 2. The patient continues to lie in a stuporous condition. There is definite paralysis of both deltoids this morning. The patient can move both legs, but the right less than the left. Knee jerk is absent on both sides this morning and the Achilles reflexes are also absent. The eyes show nystagmus easily and are still drawn chiefly to the right. The waxy or mask-like lack of facial expression continues.

October 3. The child is brighter this morning, but still lies sleepily with the head on the right, and the eyes drawn to the right. She can move them incoördinately toward the left, but there is a definite paresis of the right internal and left external recti. The temperature has dropped rapidly in the past 36 hours.

Spinal fluid: 25 c.c. of clear limpid fluid, pressure very slightly increased; cells, 112 per c.mm.; polymorphonuclears, 7 per cent.; mononuclears, 93 per cent., globulin, very slightly +; sugar, +.

October 4. The patient talks this morning and answers questions intelligently. She lies still, however, with head and eyes drawn to the right. Sometimes one gets the impression that she does not see. The temperature curve continued downward yesterday to 96.8° F., but this morning it has risen to 100° F. and now is normal again.

October 5. The child does not seem so well today. She lies more on her side with head retracted and drawn far to the right. The eyes also are still drawn to the right. She tries to avoid manipulation of the lower extremities. There is definite weakness, if not paralysis, of the quadriceps group of the right side. The leg cannot be extended on the thigh.

October 6. The child lies on her side with back arched, legs extended, and neck retracted. She moans constantly when approached. The neck is resistant. The eyes are usually turned to the right. The child cannot be made to respond at all. The arms are apparently weak. There is paralysis of the flexors of the right thigh. The child looks decidedly sick and her appearance suggests meningitis.

October 10. Spinal fluid: 20 c.c. of clear watery fluid, pressure slightly increased; cell count, 5 per c.mm.; mononuclears, ++; globulin, slightly +; sugar, +. Blood count: leukocytes, 11,200; differential count: polymorphonuclears, 52 per cent.; lymphocytes, 35.5 per cent.; large mononuclears, 7.5 per cent.; transitionals, 3 per cent.; mast cells, 0; eosinophiles, 1.5 per cent.; stimulation form, 0.

October 11. The patient is brighter and notices her surroundings, but is still irritable. There is no advance in paralyses. There is definite power now in the right quadriceps. This represents either a return or possibly an error in the finding of October 5.

October 12. Spinal fluid: 12 c.c. of clear limpid fluid; cells, 35 per c.mm.; mononuclears, +; globulin, very slightly +; sugar, +.

October 14. The child is much brighter. She is slightly propped up on pillows this morning and looks about with considerable interest.

October 20. Spinal fluid: clear colorless watery fluid, pressure not increased; cells, 12 per c.mm.; mononuclears, +; sugar, +; globulin, +.

October 23. The patient continues to improve, but she is still a little irritable, and there is pain when the spine is flexed. The left knee jerk is present, the right very indefinite. There is good power in the left leg, much less in the right.

CASE 9.

Dora O., age, 4 years. New York City. Admitted, September 30, 1911. Discharged, November 23, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The patient has always been well, though she has been a delicate child.

Present Illness.—On Wednesday, September 27, three days ago, the child was perfectly well and cheerful. She seemed well on Thursday also, until evening, when she vomited. Later in the night she was put on the toilet, left alone for a few minutes, and fell to the floor. She was put to bed yesterday morning. At that time she could not stand, though she could move her legs. This morning the father noticed that the left lower extremity was much worse than the right. She has had no convulsion and has not had any noticeable sweating.

Physical Examination.—The patient is a well nourished little girl who lies quietly and dozes when undisturbed. She objects to being handled and cries in an annoyed way on physical examination. *Eyes.*—Pupils are equal and react normally. Ocular motions are normal. *Ears.*—Normal *Nose.*—The right inferior turbinate is red and swollen. There is no discharge. *Mouth.*—Lips are dry. The buccal mucous membrane is red. Pharynx is somewhat reddened. Tonsils are not very large. The tongue is heavily coated with a whitish fur through which the red points of the papillæ can be seen. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are palpable. *Neck.*—The neck is stiff, attempts at flexion are resisted. The anterior muscles are not weak. *Back.*—The back is held rigid to prevent flexion. It hurts the patient to sit up. *Thorax.*—Well formed, respiratory movements are normal. *Lungs.*—Clear. *Heart.*—Somewhat rapid, otherwise normal. *Abdomen.*—Soft, not distended, no tenderness, no masses. *Liver.*—Not enlarged. *Spleen.*—Not enlarged. *Extremities.*—Upper: no weaknesses found. Lower: there is general weakness or possibly lack of willingness to move the right lower extremity. When prodded it is finally drawn up and moved in such a way that no definite paralyses can be made out. There is practically complete paralysis of the whole left lower extremity, except for power to flex the toes. There is also a little power left in the iliopsoas. There is definite foot drop. Knee jerks are slightly active on the right (belly of vastus internus twitches), absent on the left. Achilles reflex is

active on the right, and absent on the left. Babinski test gives plantar flexion on both sides. Kernig's sign: there is practically no resistance of a spasmodic nature on either side. Marked pain is caused on both sides by the manipulation, especially towards the end. *Temperature.*—102.4° F.

September 30. Spinal fluid: 25 c.c. of clear fluid, pressure not increased; cells, 133 per c.mm.; polymorphonuclears, 6 per cent.; mononuclears, 94 per cent.; globulin, 0; sugar, slightly +. Blood count: leukocytes, 20,000; differential count of 200 cells: polymorphonuclears, 49 per cent.; lymphocytes, 25 per cent.; large mononuclears, 26 per cent.; transitionals, 8 per cent.; basophiles, 0; eosinophiles, 1 per cent.; stimulation form, 0. *Temperature.*—101° F.

October 6. The temperature is normal. The child is very bright and lively, laughing and playing. Both legs are very tender to pressure and painful on movement. There is distinct tenderness below Poupart's ligament. The pain is so great that she can scarcely be induced to try to move the legs. The right leg does not seem to be paralyzed, but she will only move it a little. The left leg seems paralyzed except for the flexors of the toes.

October 19. The child is rather hysterical, laughs too easily and cries too easily. There is no improvement in paralysis of the lower extremities.

Spinal fluid: clear colorless watery fluid, no increased pressure; cells, 3 per c.mm.; mononuclears, +; globulin, slightly +; sugar, +.

October 24. The pain is less and the nervousness is less marked. There is no return of motion in the left leg. The quadriceps on the right is paralyzed.

October 30. The child still has pain on movement of the left leg and foot. There is no retrogression of the paralysis of the left leg. The psychic condition is about the same. She laughs hysterically and changes quickly from laughter to crying.

November 6. Spinal fluid: clear, colorless, and watery; pressure not increased; cells, 3 per c.mm.; mononuclears, +; globulin, normal; sugar, +.

November 7. The patient is in much better general condition. There is no improvement in paralysis of the left lower extremity. The right lower extremity has apparently regained complete power.

CASE 10.

Americo B., age, 2 years. West New Rochelle, N. Y. Admitted, October 6, 1911. Discharged, October 29, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The child has always been perfectly well.

Present Illness.—On Sunday, October 1, five days ago, the child seemed not quite so well as usual and vomited twice. The next day he was feverish and stayed in bed. Since that time he has been in bed. Four or five days ago when he had the fever, he also had slight convulsions. He has not cried much and has had no cough. Bowels and urination have been normal. He has been very sleepy. Has eaten well. Patient has not seemed very tender until today, when he has objected to being handled. Yesterday morning it was noticed that he could not move the left arm, which seemed tender. The other limbs have moved normally.

Physical Examination.—The patient is a well built, strong looking child,

lying on his back. He is quiet, does not cry, and is quite dull. He cannot be made to take an interest in anything. The skin is cool and rather dry. There is no rash. *Eyes.*—Pupils are small, equal, regular, and react to light. There is no strabismus. *Ears.*—No discharge or tenderness over mastoids. *Nose.*—No discharge or excoriation. *Mouth.*—The lips are dry, the tongue slightly coated. Teeth are dry and in good condition. Tonsils are slightly enlarged, not reddened. *Neck.*—The neck is stiff and resists flexion but is not retracted. *Superficial lymph nodes.*—Small glands are felt in the neck, axillæ, and groins. *Chest.*—Well formed, moves normally with respiration. Respiration is chiefly abdominal in type. There is no marked rosary. *Lungs.*—Clear on auscultation and percussion. *Heart.*—Not enlarged, action regular, sounds of good quality, no murmurs heard. *Abdomen.*—Slightly distended, soft, tympanitic, no masses or tenderness found. Abdomen moves normally with respiration. Abdominal reflexes are present. There is moderate tache cérébrale. *Liver.*—Flatness extends from the sixth space to the costal margin. Edge is not felt. *Spleen.*—Not felt. *Extremities.*—Upper: the right arm is normal. Left arm: movements of the fingers, flexion and extension of elbow are normal, but a little weak. There is apparently paralysis of the shoulder muscles, but the child is so drowsy that it is impossible to determine of which ones. Movement of the left arm is somewhat painful. Lower: movements are normal on both sides. Knee jerks are active on both sides. There is no Babinski reflex. Attempt to elicit the Kernig reflex on both sides causes pain, but no especial spasm. The child can support the head when sitting up. *Temperature.*—99° F.

October 7. Spinal fluid: 15 c.c. of clear watery fluid, pressure not increased; cell count, 12 per c.mm.; mononuclears, + +; globulin, slightly +; sugar, +.

Blood count: leukocytes, 9,800; differential count of 200 cells: polymorphonuclears, 64.5 per cent.; lymphocytes, 21.5 per cent.; large mononuclears, 7 per cent.; transitionals, 3.5 per cent.; mast cells, 1 per cent.; eosinophiles, 1 per cent.; stimulation forms, 1.5 per cent.

October 8. The child is still rather dull and pays no attention to anyone, but he is not at all comatose. The right arm moves normally. The left is paralyzed, apparently only in the shoulder. The legs are held stiff, flexed at the knees and thighs. They are not paralyzed, but all motion of the legs, and especially extension, is resisted as if painful. The right knee jerk is not obtained. The right Achilles reflex is barely obtainable. The left knee jerk is just present, left Achilles reflex is normal. There is no hyperesthesia to pressure.

October 14. The child remains in about the same condition, fairly bright, but rather irritable and whining. There is no change in the paralyses, the left upper arm only is affected. Movements and reflexes of the legs are present. There is pain on flexion of the neck, and there is resistance to flexion. There is also pain in the legs. When one attempts to handle the legs, the child flexes them sharply at the knee and resists strongly all attempts to straighten the leg, even attempts to straighten the leg when the hip is not flexed. There is no definite pain on pressure.

October 19. The patient is much less irritable. He can sit up alone and walk. He apparently has plenty of strength in the legs, but his sense of equilibrium is faulty. The paralysis of the left arm remains unchanged.

CASE II.

Moses F., age, 20 months. Brooklyn, N. Y. Admitted, September 18, 1911. Discharged, November 12, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives at Varrett Street, Brooklyn, in a house in which there are two families on a floor. There are seven other children on the same floor. Two months ago, a family with sick children had been dispossessed in this house. The nature of the sickness of those children is unknown. There are no pet animals in the family, but the usual parasites. The child had never been out of the city of Brooklyn.

Past History.—The child has always been strong and healthy, except that, according to the mother, he had pneumonia two weeks ago.

Present Illness.—Friday, September 8, ten days ago, he was perfectly well. On Saturday, September 9, the mother noticed a fever, which continued for the next eight days. The child vomited after taking medicine in the second week. Thursday, September 14, there were some choreic motions of the hands and head. On that evening, he could not move his right hand. The next morning he could not move the other hand. There was no weakness. The child has been irritable and crying and has not slept well. He complains of pain when handled. The bowels have been constipated but moved by enema.

Physical Examination.—The child lies comfortably on his back. He seems fairly bright and responds readily to stimulation. He is irritable on being handled. His legs are drawn up. At times the child has a loose cough. The head is well formed and the fontanelles are closed. *Eyes.*—There is no ptosis. The scleræ and conjunctivæ are clear. The pupils are not dilated, and both react to light, but the left pupil is slightly larger than the right. *Ears.*—There is no mastoid tenderness. *Nose.*—There is no discharge. *Mouth.*—The lips and mucous membranes are of good color. The tongue is red, moist, and has no coat. The pharynx is injected. The tonsils are visible but not large, and there is no exudate. *Neck.*—The anterior muscles seem weak. He holds his head erect, but it is unsteady, with a tendency to drop back. There is no retraction of the neck. There is marked overaction of the sternomastoids which apparently support the entire weight of the head. *Superficial lymph nodes.*—The glands in the right posterior triangle are palpable. The left axillary and both inguinal groups of glands are palpable and shotty. *Chest.*—It is well formed, symmetrical. The breathing is entirely costal in type. Expansion is fair, and slightly more on the left than on the right. The ribs move well. The accessory muscles of respiration are not brought into play. There is no apparent descent of the diaphragm. There is retraction of the abdomen on inspiration. *Lungs.*—The right side is resonant in front and back on percussion. Percussion note in upper front on left side is high pitched, in lower axilla and back it is somewhat flattened. The right side is clear on auscultation. No rales are heard over the area of impaired resonance on the left. The breath sounds over this area are somewhat more distinct than over the corresponding area on the right side. The quality, however, is normal. *Heart.*—Negative. *Pulse.*—Regular, quality good. *Abdomen.*—It is retracted on inspiration. There is no descent of the diaphragm. Abdomen is soft, no masses or tenderness. Superficial reflexes are weak, but present on both sides. The testicular reflex is active. *Extremities.*—Arms: complete

flaccid paralysis of whole right arm. Complete flaccid paralysis of left upper arm and shoulder muscles. Extension of the wrist and fingers is somewhat stronger than flexion. Brachial reflex absent on both sides. Legs: strong and can be moved in all directions. The knee jerks are active on both sides. Achilles reflexes are not obtained, Babinski reflexes are absent. Kernig's sign is absent. *Temperature.*—98.8° F.

September 18. Spinal fluid: 30 c.c. of clear limpid fluid; cells, 8 per c.mm.; globulin, very slightly +; sugar, +. Blood count: leukocytes, 20,100; differential count of 200 cells: polymorphonuclears, 45 per cent.; lymphocytes, 40.5 per cent.; large mononuclears, 0; transitionals, 9.5 per cent.; basophiles, 1 per cent.; eosinophiles, 3.5 per cent.; stimulation form, 0.5 per cent.

September 20. Percussion of lower left lung is unchanged. No rales are heard. The paralysis remains the same. The breathing is purely costal in type. The intercostals are strong.

September 25. Spinal fluid: 24 c.c. of slightly blood-tinged fluid; the specimen for the tests is clear; cells, 4 per c.mm.; globulin, very slightly +; sugar, +. Blood count: leukocytes, 25,600; differential count of 200 cells: polymorphonuclears, 59 per cent.; lymphocytes, 33.5 per cent.; large mononuclears, 0; transitionals, 7.5 per cent.; basophiles, 0; eosinophiles, 0; stimulation form, 0.

September 30. The diaphragm still does not act, but the patient seems perfectly comfortable with the intercostals acting alone.

October 2. Spinal fluid: 18 c.c. of clear fluid; cells, 3 per c.mm.; globulin, 0; sugar, +.

October 24. For the past week the patient has been walking alone. He carries his head far forward with his face a little down, so that he looks up under his eyebrow to see where he is going. The head is held also a little to the right. The trapezius seems to be doing all the work of holding the head up. This gives a peculiar sloping line from head to shoulder and makes the neck look very short and thick. There is no return of power in the arms.

October 30. The child is improving somewhat. He walks very well. He still holds his head tilted to the right. He tries to use his hands and can now pick up objects from the floor with his left hand, but there is little improvement in the arms.

November 12. The child is in excellent general condition. The heart, lungs, and abdomen are clear. The child walks very well and can even run a little. The pupils are equal, regular, and react normally. The neck, back, and abdominal muscles are strong. There is apparently some overdevelopment of the trapezius which supports the head. The pectorals are atrophied. There is a large fold of fat just inside the axillæ, over the pectorals, and a heavy collar of fat around the neck. The legs move normally. Both knee jerks are exaggerated. There is no true ankle clonus. No Babinski or Kernig's signs. The cremasteric and abdominal reflexes are present. The right arm and hand are completely paralyzed from shoulder to fingers. The left shoulder and apparently the extensors of the hand are paralyzed. The only movements possible are flexion and extension of the thumb. He has a remarkably strong grip with the left hand.

CASE 12.

John B., age, 3 years. New York City. Admitted, July 18, 1911. Discharged, August 20, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a sixteen family tenement. The home is clean and has plenty of air. As far as is known, there are no sick children in the house, and the child has come in contact with no one who is lame or paralyzed.

Past History.—He has always been well except for two attacks of measles, the last one a year ago.

Present Illness.—Two days ago, on July 16, the child was perfectly well. He usually takes a nap in the afternoon, but on this day he went to sleep in the morning. When he woke up at noon, he started to play, and one of the other children noticed that he could not lift his hand. However, in the afternoon he was well enough to play. He did not complain of pain, has not vomited, has not sweat, and has not been feverish. He has not been especially restless. Yesterday he was playing but could not use his arm. He has had no headache. Appetite has been poor. Bowels and urination normal. The arm has not become any worse. Face has been unaffected.

Physical Examination.—The patient is a well built little boy lying quietly half asleep. He takes little notice of his surroundings and is not easy to rouse. The skin and mucous membranes are of good color. *Eyes.*—The eyes move normally. The right pupil is larger than the left. The right pupil reacts to the light but the left apparently does not. *Ears.*—Normal. There is no discharge and no tenderness over the mastoids. *Mouth.*—The tongue has a grey coat and protrudes to the right. The teeth are in good condition. The tonsils are enlarged and ragged. There is no exudate, and they are not reddened. *Superficial lymph nodes.*—There are small lymphatic glands palpable in the neck, axillæ, and groins. *Chest.*—Well formed, symmetrical, and both sides move equally. *Heart.*—Normal. *Lungs.*—Normal on auscultation and percussion. *Abdomen.*—Full, soft, tympanitic throughout. It is symmetrical and moves normally with respiration. There are no masses or tenderness. Liver flatness extends from the sixth rib to the costal margin. Edge is felt 2 cm. below the costal margin. Spleen is not felt. Abdominal reflexes are not obtained. *Muscular system.*—Legs: knee jerks and Achilles reflexes are present on both sides. The other reflexes are negative. Movement of the legs: there is apparently no paralysis or weakness of either leg. Arms: the muscles of the right arm seem to be unaffected. There is a complete flaccid paralysis of the left arm and hand. The child does not move the arm or hand, and when lifted, it falls limply. Muscles of the back: there is no stiffness of the neck. Child sits up well and holds up its head. *Face.*—The right side of the face is slightly more flattened than the left. The folds of the skin are less marked on the right. The left corner of the mouth is drawn up slightly, and this becomes more marked when the child cries. The right eye slit is narrower than the left. There is apparently no weakness of the right upper lid as this can be raised normally. *Surface.*—There is a small area of herpetic eruption on the upper lip just to the left of the median line.

July 18. Blood count: leukocytes, 15,100; differential count of 200 cells: polymorphonuclears, 55 per cent.; small mononuclears, 26 per cent.; large mononuclears, 8.5 per cent.; transitionals, 1.5 per cent.; eosinophiles, 9 per cent. Red cells normal; blood platelets normal.

July 19. Spinal fluid: 20 c.c. of perfectly clear colorless fluid; pressure not increased; cell count, 37 per c.mm.; globulin, slightly opalescent, slightly +;

albumen, slightly +; sugar, +; smear, small mononuclears, 88 per cent.; large mononuclears, 10 per cent.; polymorphonuclears, 2 per cent.; a few red cells.

July 21. Left arm: it is difficult to make the child attempt movements, but this morning he can extend his fingers, flex the fingers slightly, and flex and extend the thumb. The child complains of pain in the legs, but reflexes and movements are normal.

July 23. The left pupil reacts normally.

July 25. Spinal fluid: 15 c.c. of perfectly clear watery fluid; not opalescent; no increase of pressure; cell count, 12 per c.mm.; smear, chiefly small mononuclears, too few to count; globulin, slightly +.

July 27. There is still very slight evidence of right-sided facial paralysis. The left eye slit is slightly narrower than the right. When seen this morning the child was sweating profusely about the head. The sweating was much more intense on the right side of the head, face, and forehead. Both pupils react normally to light. There is no more paralysis of dilation of the pupil. The tongue protrudes straight.

July 31. The knee jerk is active on both sides. There is hemicranial sweating. Sweating on the right side of the face is frequently observed, while the left side is practically without sweat. The left eye slit is narrower than the right. The right side of the face has a better pink color than the left. The pupils are equal, regular, and react normally.

August 20. His general condition is very good. The chest and abdomen are negative. The legs move normally. The reflexes are normal. The left eye slit is slightly narrower than the right (the lid is a little swollen). The pupils are equal, regular, and react normally. The eyes move normally. The right arm is normal. Left arm: there is complete paralysis of the shoulder muscles and of the flexors of the elbow. The child seems able to use the triceps to extend the elbow. Extensors of the hand are strong, flexors of the fingers and muscles of the thumb are weak but act fairly well. The child has had no hemicranial sweating or flushing of late. He is discharged.

CASE 13.

Victoria M., age, 4 years. Sandy Hook, N. J. Admitted, July 15, 1911. Discharged, September 9, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a large house at Sandy Hook. There are three families in the house, but each has a separate entrance. The house is twelve years old and clean. There are no other children in the house. There is no one lame or paralyzed in the vicinity. The children have not been away from Sandy Hook since last October, and neither children nor parents have come in contact with anyone who is lame or paralyzed.

Past History.—The child was a normal baby. She is thought to have had scarlet fever four months ago, but the diagnosis is uncertain.

Present Illness.—On June 13 the child complained of pain in the stomach and vomited twice. She was very restless, slept poorly, and sweat a great deal, especially around the head. For the next two weeks she was in bed about half the time and was in poor health. She was slightly feverish and dull. She complained of no pain except in the stomach. On June 23 she complained of pain in her right foot and her mother noticed that when she got up in the morning she

limped and could not use her legs well. In the afternoon it was seen that she did not use her hands normally. The next morning she was taken to the Presbyterian Hospital. About a week before the onset of the paralysis, the mother noticed that while she was sleeping, her breathing was not like that of the other children. When breathing, her chest moved much more than normally, and her abdomen moved much less.

Physical Examination.—The patient is a fairly well built little girl, lying on her back. The skin is clear. *Eyes.*—The eyes move normally, and the pupils react normally. *Ears.*—Normal, no discharge. No tenderness over the mastoids. *Mouth.*—The tongue is clean. The tonsils are somewhat enlarged; the throat is clean. *Superficial lymph nodes.*—There are small glands in the neck, axillæ, and groins. *Chest.*—Respiration is very irregular and labored. After one or two shallow, quiet respirations, the child takes a deep forced inspiration which is associated with dilatation of the alæ nasi. There is marked retraction of the abdomen and flaring of the ribs. With each respiration, the sternomastoid muscles become very tense, and the front of the chest is raised to a marked degree. The left side of the chest is more prominent than the right. Respiration is for the most part of a very jerky character and is at times like hiccough. This is, however, much diminished when the child is lying quietly and has stopped sobbing. *Heart.*—Negative. *Lungs.*—Normal throughout on auscultation and percussion. *Abdomen.*—Level, soft, tympanitic, no masses or tenderness. At times, especially on expiration, there is marked bulging of the left upper quadrant. Abdominal reflexes are not obtained. *Liver.*—Flatness extends from the sixth intercostal space to the costal margin. Edge is not felt. *Spleen.*—Not felt. *Muscular System.*—Legs: knee jerks, Babinski and Oppenheim reflexes are absent on both sides. Achilles reflex is barely obtainable on both sides. Extension of the legs causes marked pain so that Kernig's sign cannot be tested. Movements: flexion of the thighs is possible. There is toe drop and apparently paralysis of the quadriceps and the anterior tibial muscles. Posterior tibial muscles on both sides are unaffected. Movements of the arms are normal. The head flexes without pain. The child can sit up and hold her head up but is apparently rather weak. She complains of pain in both thighs. *Temperature.*—100° F.

July 16. The child breathes quietly when let alone, but inspiration is always associated with retraction of the abdomen. On expiration there is bulging of the upper half of the abdomen. The muscular tone is good.

July 18. The nurse noticed yesterday that the patient was unable to hold a piece of bread well in her hands. Arms: movements of upper arms, and flexion and extension of forearm are normal. Dorsal extension of hand at wrist is impossible on both sides. Flexion of the fingers is fairly complete but weak on both sides.

July 19. The right side of the face shows some flattening, and the left corner of the mouth is drawn up. The left eye slit is narrower than the right. The tongue protrudes straight. The voice is very harsh, squeaky, and high pitched.

July 23. The chest and abdomen are clear. Paralysis of the diaphragm persists.

July 25. Spinal fluid: 18 c.c. of clear watery fluid, not opalescent; no increase of pressure; partly bloody; cell count, 163 per c.mm.; smear, small mononuclears,

87 per cent.; large mononuclears, 9 per cent.; polymorphonuclears, 4 per cent.; globulin, slightly +.

July 31. Spinal fluid: a few cubic centimeters of perfectly clear colorless fluid; no opalescence; no increase of pressure; cell count, 979 per c.mm.; many large phagocytic cells; smear, small mononuclears, 68 per cent.; large mononuclears, 16 per cent.; endothelial cells, 16 per cent.; no polymorphonuclears; many degenerated cells in the smear, apparently endothelial cells; globulin, slightly +. Temperature, 100° F.

August 2. Spinal fluid: 10 c.c. of perfectly clear watery fluid; no opalescence; pressure very low; cell count, 154 per c.mm.; a few phagocytic cells; smear, small mononuclears, 96 per cent.; large mononuclears, 4 per cent.; no blood; globulin, slightly +, opalescent; few small flocculi.

August 5. There is gradual increase of power in the quadriceps on both sides. Both knee jerks were elicited this morning, the left being more active.

August 8. Spinal fluid: 8 c.c. of clear watery fluid; pressure too low to measure; cell count, 31 per c.mm.; smear, large percentage of mononuclears; globulin, very slightly +, slightly turbid, within normal limits; sugar, +.

August 15. The diaphragm is now acting absolutely normally. The abdominal muscles act normally, and the reflexes are normal. Both knee jerks and both Achilles reflexes are obtained normally, there is no ankle clonus. The movements of both legs are normal, and fairly strong. She is walking and gets along well. The muscles of the neck are still weak (?), the head tends to fall backwards. The extensors of the hand and muscles of the thumb show little improvement.

CASE 14.

Gladys B., age, 6 months. New York City. Admitted, October 27, 1911. Discharged, November 11, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a tenement. There are eighteen families in the building. The building is not old, and it is very airy and clean. There are no cats or dogs in the home; occasionally there are cockroaches. Max G. (a cousin of this child, and now in this hospital with poliomyelitis) lives on the ground floor of this building. There is very close contact between these two families, and Max plays continually with the B. children. On Tuesday, October 17, when Max was sick in bed, the baby was brought in and laid on a pillow on his bed. Shortly after that, Max was taken to the hospital. Since that time Gladys has not been in the G. home.

Past History.—Negative.

Present Illness.—On the morning of Saturday, October 21, six days ago, the baby was perfectly well when bathed. In the afternoon she vomited twice and had "high fever." The doctor thought it was angina and middle ear trouble. The next day she was feverish and very sick. There has been no vomiting since the first day of her illness. She has had no convulsions and has not sweat at all. Her bowels have been constipated. Urination has been normal. During her illness the child has taken no interest in anything, and has slept most of the time. She has wanted to lie quietly in bed, and apparently has objected to being handled. She has taken her nourishment well. Two days ago, October 25, the mother noticed that the child did not use her left hand. Even the day before,

the mother noticed that the child did not use her hands or her feet much. Yesterday she could move only the fingers of the right hand. The left arm and right leg were also paralyzed. The child looks brighter today and has less fever.

Physical Examination.—The patient is a well built and strong looking baby. Sensorium is clear. The neck resists flexion. There is no retraction. The skin is clear except for a little scaling on the upper lip. The anterior fontanelle is open, but not bulging. The face is symmetrical. *Eyes.*—Pupils are equal, regular, and react to light. Ocular movements are normal. There is no strabismus or nystagmus. *Ears.*—There is no discharge or tenderness. *Mouth.*—Tongue is clean. Tonsils are slightly enlarged and rather ragged. There is no redness. Pharynx is normal. *Superficial lymph nodes.*—Glands in the neck are palpable. *Chest.*—The chest is symmetrical and moves well with respiration, but with inspiration there is a very definite retraction and descent of the anterior chest wall. This is associated with a marked raising of the abdominal wall on inspiration. There is no special activity of the accessory muscles of respiration. Respiration is rapid, but not labored. Pressure over the abdomen causes great distress, but pressure over the chest does not affect respiration. *Heart.*—Normal in size. Regular. Sounds are of good quality. *Lungs.*—Clear on auscultation and percussion, except for a few scattered moist rales in both backs. *Abdomen.*—Full, soft, and tympanitic. There are no masses or tenderness. Abdominal reflexes are present. When the child sits up the abdominal muscles seem to lack tone. There is definite tache cérébrale. *Liver.*—Normal area of flatness. Edge not felt. *Spleen.*—Not felt. *Extremities.*—Right arm appears normal. Left arm: flexion and extension of fingers and wrists are normal; flexion and extension of the elbow are weak, and it is difficult to determine whether or not it is completely paralyzed. Lower extremities: right leg is paralyzed completely, except for the extensors of the toes and foot (anterior tibial and peroneal). In the left leg there is weakness of the quadriceps. Knee jerks, Achilles and Babinski reflexes are absent. Kernig's manipulation produces pain on the right, but not on the left side. *Temperature.*—99° F.

October 27. Spinal fluid: 15 c.c. of clear limpid fluid; pressure not increased; cells, 25 per c.mm.; mononuclears, + +; globulin, normal; sugar, +.

October 31. Paralysis of the intercostal muscles persists. There are no rales in the lungs. The general condition is excellent.

Spinal fluid: 15 c.c. of clear watery fluid; pressure slightly increased; cells, 5 per c.mm.; mononuclears, +; sugar, +; globulin, +.

November 6. Spinal fluid is clear, colorless, and watery; cells, 5 per c.mm.; mononuclears, +; globulin, very slightly +; sugar, +.

November 11. The child is in good condition. He has had a loose cough for some time and this persists slightly. The chest and abdomen are clear. His respiration is entirely abdominal. There is still some retraction of the thorax on inspiration, but it is not so marked as on admission. Paralysis of the intercostals persists. Both hands, the lower arms, and the elbows move normally. There is weakness of the shoulders, especially of the deltoids. The arms can only be raised incompletely, but still there is very good motion in every direction. The legs move normally. The knee jerks are both present. Achilles reflexes are not obtained. There is no Kernig's sign or Babinski reflex. The abdominal reflexes are normal. The residual paralysis consists of weakness of both shoulders and intercostals.

CASE 15.

Louis G., age, 3 years. New York City. Admitted, July 31, 1911. Discharged, September 3, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family live in a tenement with nineteen other families. The house is not very old, is fairly clean, and has good air. There are no bedbugs or other parasites found in the home, and no dogs or cats belong to the family. They know of no other sickness in the house or neighborhood and of no lame or paralyzed children or any possibility of contact.

Past History.—The child was always a healthy baby, nursed for eighteen months. He had a "convulsion" about every six weeks "when the teeth came out," but he has had none for the past six months. Six months ago he had measles, followed by whooping cough, the latter lasting five or six weeks.

Present Illness.—Two weeks ago yesterday, July 16, the child was well in the morning. At one o'clock in the afternoon the father found him very feverish, and sent for a doctor. The next day the child was worse, and still feverish. He had no convulsions, no vomiting, and no cough, but was very restless and sweat a great deal. His appetite was poor. After three days the child got better, but stayed in bed because his neck was weak, and he could not hold up his head. Eight days ago, July 23, the father says that he complained of pain in the left foot, and it was noticed that he could not move either foot. Since the first three days he has had no fever. For the past eight or nine days he has had a little cough, but no expectoration. The bowels have been constipated and appetite very poor. The arms have not been affected. The child has had no trouble with respiration, and was doing well until yesterday afternoon at three o'clock, when the parents noticed that he was having difficulty in breathing. Respiration was rapid and associated with much movement of the belly wall. Since then he has become much weaker, has not slept, and has eaten very little. He has had more cough and has cried all night. He has not seemed to have pain or tenderness since the onset, when he had pain in the neck and foot.

Physical Examination.—The patient is a well developed, very sick looking little boy. Sensorium seems clear. The lips are pale and have dry crusts on them; no herpes. The skin is hot and dry and rather grey. On the chin are two pinhead sized petechial spots, no rash, no bites. The head is well shaped and the face symmetrical. Child swallows milk well. *Eyes.*—Movements are normal, no strabismus. Pupils are equal, regular, about 4 mm. in diameter. They contract in bright light, but do not dilate well. The right eye slit is a trifle narrower than the left. On looking downward the sclera is seen above the left cornea. *Ears.*—No discharge or tenderness. *Nose.*—No discharge. *Mouth.*—The teeth are in good condition. Tongue protrudes straight, and has a thick grey coat. The tonsils are much enlarged, and rather ragged. No redness or exudate. *Neck.*—The neck resists flexion and there is slight retraction. *Superficial lymph nodes.*—The glands are not especially enlarged, but there are a few small ones in the neck and groins. *Chest.*—Symmetrical, well formed, costal angle is wide. Respiration is rapid. The neck muscles come into play actively, the sternomastoids stand out with each inspiration, and the alæ nasi dilate with respiration. Respiration is wholly of the abdominal type. The chest hardly moves at all with respiration, the only movement being a retraction of the lower ribs and of the lower intercostal spaces on inspiration. The abdominal

wall bulges forward on inspiration, and is retracted on expiration. Breathing is rapid and jerky, expiration is forcible. The expiratory sounds follow the retraction of the abdomen at a considerable interval. *Heart.*—Not enlarged, sounds obscured by rales, sounds of embryonic type. *Lungs.*—No dullness, no pure bronchial breathing, but both lungs are everywhere full of coarse moist rales. Respiration is harsh and loud. *Abdomen.*—Full, soft, tympanitic, no masses or tenderness. The left side is fuller than the right. Abdominal walls are very lax and bulge forward with inspiration. There is apparently a paralysis of the muscles of the abdominal wall. Abdominal reflexes are not obtained. *Liver.*—Flatness extends from the sixth rib to the costal margin, edge not felt. *Spleen.*—Not felt. *Muscular system.*—The arms move normally but there is apparently slight weakness of the right. Both legs lie limply on the bed and the child neither moves them nor resists passive motion. Movement of the flexors of the toes on the right is noted on plantar stimulation, but otherwise the legs are paralyzed. Knee jerks, Achilles, Babinski, Oppenheim, and Kernig reflexes are absent on both sides. The attempt to elicit the Kernig's sign is painful. It is impossible to determine about the back muscles, but they seem quite strong as the child is made to sit up. The child can hold his head up, but with difficulty. No pain or hyperesthesia is apparent except on attempting to flex the neck and in trying to elicit Kernig's sign. The child is very sick and it is impossible to determine the limits of paralysis accurately.

July 31. Blood count: leukocytes, 19,700; differential count of 200 cells; polymorphonuclears, 84 per cent.; small mononuclears, 6.5 per cent.; large mononuclears, 6.5 per cent.; transitionals, 3 per cent.; mast cells, 0; eosinophiles, 0.

August 4. Spinal fluid: 20 c.c. of perfectly clear watery fluid, not opalescent; pressure 100–110 mm.; cell count, 29 per c.mm.; differential wet count: 98 per cent. mononuclears; 2 per cent. polymorphonuclears; globulin, + (flocculent precipitate).

August 5. The chest is more free of rales. All the reflexes of the legs are still absent.

August 8. The chest is practically clear of rales. The abdominal and back muscles are strong. The neck muscles are apparently strong, but on sitting up the child has a tendency to let his head hang backwards.

August 19. The chest is clear. The child cries as soon as anyone tries to touch him or move his legs. He seems to have a good deal of tenderness still. The legs are the same as yesterday. Both knee jerks and Achilles reflexes are absent.

September 3. The child is very bright and active. He is able to sit up alone. The movements of the head and neck are good. The abdominal reflexes are active, and there is no sagging of the abdominal walls when the child sits erect. The spine does not deviate to either side. The knee jerks are absent on both sides; Achilles reflexes are absent; there is no ankle clonus, but strong plantar flexion of toes on the left on plantar stimulation, and wink-like response on right. Movements of the right leg are absent below the hip. The left thigh can be weakly flexed, extended, and rotated outward. There is no apparent power of abduction. Flexion and extension of the right lower leg are absent, the left lower leg can be rather strongly flexed and extension of the foot is good. The

toes on the left side can be both flexed and extended. The ribs move well on respiration, slightly more on the left than on the right side. The patient goes home today.

CASE 16.

Hyman W., age, 21 months. Brooklyn, N. Y. Admitted, September 22, 1911. Discharged, November 26, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis and acute bronchitis.

Family History.—Negative. Habitat: the family lives in a four family house on Willoughby Avenue, Brooklyn. They have four rooms on the first floor The light in the bedrooms is bad, in the other rooms it is fair. There is no similar illness in the house. The child has played with other children of the street and house. There are flies and mosquitoes in the house, no roaches or waterbugs, but some bedbugs.

Past History.—The patient has always been a healthy child.

Present Illness.—On Sunday morning, October 15, five days ago, the child was perfectly well. In the afternoon he had two attacks of vomiting. The next morning, Monday, he had a temperature of 103–104° F., but was up and walked about during the day. On Tuesday, two days after the onset, paralysis of both legs was noticed and weakness of the right arm. On Wednesday, the child had some difficulty in swallowing. He has had no dyspnea or convulsions. Child has not been very irritable. For two days he has had pain when the head was raised, and on sitting up the head dropped backward. There has been no rigidity or retraction of the neck. He has had no discharge from the ears, no cough. Bowels moved last on Wednesday after enema and castor oil. There has been no retention of urine.

Physical Examination.—The child lies quietly in bed sleeping. On being awakened he responds slowly to stimulation and is drowsy and dull. He cries weakly on being handled. The skin is moist and warm. There is a slight degree of dyspnea. *Head.*—Well formed, slight frontal prominence. Fontanelles are closed. *Face.*—Symmetrical in repose, drawn slightly to the left on crying. *Eyes.*—Apertures are equal. Scleræ and conjunctivæ are clear. External ocular movements are good, no paralysis of orbicularis. Pupils are equal, not dilated, react slightly to light. *Ears.*—No tenderness or discharge. *Nose.*—No discharge, nares patent. *Mouth.*—Lips and mucous membranes are of fair color. Tongue has a heavy coat, muscles are intact. Pharynx is injected. Tonsils are not enlarged, and there is no exudate. *Neck.*—No retraction. There is slight resistance to flexion. On sitting up the head falls back and to the right. There is paralysis of the anterior neck muscles and sternocleidomastoids. *Superficial lymph nodes.*—The glands on both sides of the neck are palpable and shotty. *Chest.*—Well formed and symmetrical. There is slight flaring of the costal border. Breathing is diaphragmatic in type. Ribs do not move. There is retraction of the bony wall on inspiration. There is slight action of the accessory muscles of respiration. The diaphragm descends, and seems fairly strong. Respiration is slightly rapid, short, and jerky. *Lungs.*—Clear on ausculation and percussion. *Heart.*—Normal. *Abdomen.*—The abdomen moves well with respiration. Walls are relaxed. There is no bulging in the flanks, no masses or local tenderness. Superficial abdominal reflexes are absent. *Extremities.*—Upper: the right arm has very slight power of flexion of the fingers, otherwise it is in a

state of complete flaccid paralysis. The deltoid and posterior arm muscles are paralyzed on the left. Other movements are possible but weak. Lower: the right thigh can be extended with a strong tendency to outward rotation. The toes and foot can be extended. On the left side there is slight power of outward rotation with slight extension of the thigh. Otherwise flaccid paralysis is complete on both sides. There is pain on full flexion of the leg but no Kernig's sign. Knee jerks, Achilles reflexes, and Babinski test are all absent. *Temperature.*—99.6° F.

September 22. Spinal fluid: 20 c.c. of perfectly clear fluid, pressure slightly increased; cells, 5 per c.mm.; mononuclears, + +; globulin, 0; sugar, +. Blood count: leukocytes, 17,100; differential count of 200 cells: polymorphonuclears, 38 per cent.; lymphocytes, 49 per cent.; large mononuclears, 1.5 per cent.; transitionals, 11 per cent.; basophiles, 0; eosinophiles, 0; stimulation form, 0.5 per cent.

September 25. The alæ nasi are working this morning a little more than before. The child shakes his head from side to side frequently. Evidently this is the only way of attempting to escape from the discomfort of breathing and general sense of helplessness.

September 26. Spinal fluid: 30 c.c. of clear limpid fluid, pressure very slightly increased; cells, 15 per c.mm.; globulin, ±; sugar, +.

September 27. Spinal fluid: 10 c.c. of clear watery colorless fluid, pressure not increased; cells, 4 per c.mm.; all mononuclears; sugar, +; globulin, slightly +.

September 28. The patient continues to breathe only with the diaphragm. There is no return of power in the lower extremities or arms. The child sleeps little at night but dozes from time to time during the day.

October 3. Blood count: leukocytes, 17,200; differential count of 200 cells: polymorphonuclears, 49.5 per cent.; lymphocytes, 39 per cent.; large mononuclears, 6.5 per cent.; transitionals, 3.5 per cent.; basophiles, 0; eosinophiles, 1 per cent.; stimulation form, 0.5 per cent.

October 4. The patient has improved markedly in the past three or four days. He whines less and looks stronger and brighter. He breathes strongly with the diaphragm.

Spinal fluid: 14 c.c. of clear fluid, pressure not increased; cells, 2 per c.mm.; globulin, +; sugar, +.

October 18. During the past week the patient has seemed somewhat brighter. There is, however, no change in his paralysis. The neck is apparently unable to hold up the head. There is no return of costal respiration.

October 20. Spinal fluid: clear, colorless, and watery; cells, 2 per c.mm.; mononuclears, +; globulin, slightly +; sugar, +.

November 2. The child really makes no progress. He is a miserable, sick child. The neck muscles are still so weak that the head drops backward when he is sitting up. The back is very weak. The power of the right arm is completely gone except for flexion of the fingers. The left upper arm is paralyzed. Breathing is practically wholly diaphragmatic. The intercostals are paralyzed. The child still has pain on moving the legs. He does not look so bright or so well as he did two weeks ago. The chest is clear except for a few rales in the back.

November 3. Spinal fluid: clear, colorless; cells, 3 per c.mm.; mononuclears, +; sugar, +; globulin, very slightly +.

November 17. Yesterday morning there was a beginning bronchitis on both sides. In the evening both lungs, front and back, were full of coarse, moist rales. No rise in temperature was noted. Respiration was rapid and wholly abdominal. Complete paralysis of the intercostals persists. The chest has seemed to become smaller and more shrunken, an appearance probably due in part to atrophy of the pectorals and intercostals, associated with the soft protuberant belly. This morning the child looks better. His temperature is 100° F., and the rales are not quite so numerous.

November 19. The condition remains the same. Both sides of the chest are full of coarse moist rales. The heart action is good. There is no especial rise of temperature.

November 21. The temperature is still normal. The rales have cleared up remarkably, and the chest is almost clear. The child looks much better.

November 26. The patient continues to do well. This morning there are a few, low pitched, coarse rales in both sides of the chest near the bases. The breathing is still entirely diaphragmatic. The abdomen is greatly distended. The patient has gained in weight and looks very fat and well. The neck is not yet able to support the head, and the back is very weak. The child goes home today.

CASE 17.

Edward G., age, 3 years and 8 months. New York City. Admitted, August 12, 1911. Discharged, August 23, 1911. Result, well. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The patient has always been healthy.

Present Illness.—Last Sunday, August 6, six days ago, the patient came to his mother and said he did not feel well and wanted to lie down. He was sleepy and complained of headache. On Monday he walked about a little, but went to bed again and has been there since. He has had no appetite, has not been irritable, and has had no tenderness. On Tuesday he either could not or would not speak, and has not spoken since. He has eaten nothing since Tuesday. He had not vomited until yesterday, when he vomited a little after taking medicine. He did not lie quietly, but threw himself about in bed. At times he has seemed almost to have convulsions. Bowels have not been regular. He has passed urine normally, but has not voided since yesterday. Urination induced by application of hot compresses. No sore throat. The mother noticed no weakness of the arms or legs, but says there is some stiffness in the neck.

Physical Examination.—The patient is a well developed, rather thin, very sick looking child. He lies quietly on his back, eyes half closed, and pays no attention to his surroundings. He cannot be roused by questions and is very dull. The skin is dry, quite warm, and clear, except for a few old scabs on the legs and arms (bites). The face is symmetrical. *Eyes.*—The eye slits are equal. Eyes apparently move normally, but it is impossible to rouse him enough to make him follow an object with his eyes. Pupils are equal, and react normally. *Ears.*—No discharge or tenderness. *Nose.*—No discharge or excoriation. *Mouth.*—The lips are covered with dry crusts, no herpes. Teeth are dry and in fair condition. Tongue has a thick grey coat. The tonsils are only a little enlarged. Tonsils, pillars, and pharynx are reddened; no exudate. The pharynx is full of grey

mucopurulent material. *Neck.*—There is very slight resistance to flexion, no retraction. *Superficial lymph nodes.*—There are firm discrete enlarged glands in the neck, axillæ, and groins. *Chest.*—Somewhat chicken-breasted, no definite rachitic rosary or Harrison's groove. Chest is symmetrical and moves normally with respiration. *Heart.*—Negative. *Lungs.*—Clear on percussion and auscultation. *Abdomen.*—Level, soft, tympanitic, no masses or tenderness, symmetrical, moves normally with respiration. Abdominal reflexes are present. Bladder is distended. *Liver.*—Flatness extends from the sixth space to the costal margin. Edge is easily felt. *Spleen.*—Not felt. *Muscular system.*—Legs: reflexes, all normal. No paralysis or weakness of leg muscles. Arms: both move equally and strongly. Back and abdominal muscles are strong. Neck muscles: the child can sit up straight, but after a short time his head falls backwards. He has very little power to straighten his head up again, and usually lets it continue to hang backwards. *Temperature.*—99.8° F.

August 12. Blood count: leukocytes, 15,400; differential count of 200 cells: polymorphonuclears, 63 per cent.; lymphocytes, 17 per cent.; large mononuclears, 5 per cent.; transitionals, 8.5 per cent.; basophiles, 0; eosinophiles, 6 per cent.; stimulation form, 0.5 per cent.

Spinal fluid: 30 c.c. of perfectly clear watery fluid, not opalescent; pressure, 330 mm.; cell count, 31 per c.mm.; globulin, slightly cloudy, within normal limits; sugar, + after heating; sodium chloride, 0.70 per cent.; smear, small mononuclears, 96 per cent.; large mononuclears, 4 per cent.

August 14. The child is much brighter, is wide awake, and will sit up alone. He does not respond well to questions and still prefers to be let alone. The neck muscles are much stronger. Movement of the head is painful but he can hold his head up well. The right knee jerk is very slight and is obtained with difficulty.

August 15. Urination has been normal since admission.

August 18. Spinal fluid: 20 c.c. of clear colorless fluid; no blood; pressure slightly increased (crying); cell count, 7 per c.mm., all mononuclears; globulin, very slight opalescence, normal; sugar, +.

August 23. Spinal fluid: 15 c.c. of perfectly colorless watery fluid; cell count, 6 per c.mm., all mononuclears; globulin, very slightly +, turbid; no flocculi; sugar, +. Eyes, ears, and throat are negative. There is no stiffness of the neck. Neck, back, and abdominal muscles are strong. Movements of the legs and arms are normal. The child walks well. He blinks a good deal, and seems to have some photophobia. The patient is discharged well.

CASE 18.

Mary H., age, 8 years. New York City. Admitted, October 2, 1911. Discharged, October 22, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative.

Past History.—The patient has always been a healthy child.

Present Illness.—Last Thursday, September 28, four days ago, the patient vomited. She had been perfectly well on Wednesday night. The doctor gave her some medicine and she stayed in bed all that day and was drowsy. She did not sweat much. Slept fairly well Thursday night. On Friday she seemed much better and asked for food, but stayed in bed. That night she was restless. Satur-

day morning her father saw her suddenly become stiff, but she did not have a convulsion. This passed rapidly, and during the day she became more and more stuporous. The doctor said that it was either typhoid or meningitis. The child was restless during the night. Yesterday morning (Sunday), and throughout the day, she was in a very stuporous condition. Yesterday evening Dr. Huber was called and made a diagnosis of infantile paralysis. About 9:00 P. M. yesterday evening, the patient could swallow only very weakly. One eye could not be closed. She was restless during the night; no sweating. She has been constipated.

Physical Examination.—The patient is a well nourished little girl, tall and slender. She lies on her back with head to the right. The right eye is closed, the left open. She is in a stuporous condition from which she can be roused by handling or by sharp questions and orders. She will perform any simple act, such as putting out the tongue, raising the eyebrows, etc. She does not become irritated during the course of the examination, but accepts it all passively. She is very apathetic. There is incontinence of urine. *Face.*—The left half of the face is completely paralyzed. The eyebrows cannot be moved, the eye cannot be shut, and when she shows her teeth the right side pulls far over. *Eyes.*—Pupils are equal, react rather sluggishly. The left eye slit is wide and cannot be closed. The ocular motions are a little incoördinate, there being a definite weakness of the right internal rectus so that an internal strabismus of the right eye is present most of the time. *Ears.*—Normal. *Nose.*—Normal. *Mouth.*—The tongue is heavily coated. There is much thick, grayish, sticky mucous in the back of the mouth. The buccal mucous membrane is red. The pharynx is very much congested; tonsils not present. *Neck.*—The neck is stiff to flexion. Anterior muscles are weak. She cannot keep the head from falling backward. Swallowing is weak. *Superficial lymph nodes.*—The posterior cervical are somewhat enlarged. Axillary and inguinal nodes are just palpable. *Chest.*—Rather long and narrow, moves only very slightly in respiration, but if the patient takes a deep breath it moves normally. *Lungs.*—Clear. *Heart.*—Normal. *Abdomen.*—Soft, flat, no tenderness, no masses. Enlargement of liver and spleen is not evident. *Extremities.*—Upper: no weaknesses. Lower: no weaknesses. Knee jerks are very active; Achilles reflexes active. On testing for the Babinski sign, there is slight rapid initial flexion and then marked extension of the great toe. Oppenheim reflex is present. Kernig manipulation is resisted actively near the end and the child complains of pain. *Temperature.*—99.6° F.

October 2. Spinal fluid: 14 c.c. of clear fluid, pressure not increased; cells, 20 per c.mm.; mononuclears, ++; globulin, very slightly +; sugar, +. Blood count: leukocytes, 12,000; differential count of 200 cells: polymorphonuclears, 75 per cent.; lymphocytes, 13 per cent.; large mononuclears, 3.5 per cent.; transitionals, 8 per cent.; basophiles, 0; eosinophiles, 1 per cent.; stimulation form, 0.5 per cent.

This evening the patient was fed by gavage. She has difficulty in managing fluid in the mouth and pharynx and has much distress from thick saliva.

October 3. The patient is still very dull, but can be roused and responds slowly to questions. Some weakness of the right deltoid seems to be present this morning. The brachial reflex on the left is present, on the right absent.

October 4. The child is markedly brighter this morning. Both eyes are open

and she is no longer stuporous. She is still apathetic. There is slight internal strabismus of the right eye. There is no improvement of the left facial paralysis. The patient can speak coherently this morning, and says she has a little pain in her left foot. The knee jerks are present, but not especially active; they are about normal. The left Achilles reflex is much more active than the right. She has no loss of power in arms or legs, though she complains of pain in the left foot. The anterior neck muscles are weak, for the patient's head drops back when the trunk is lifted forward by the shoulders. In the sitting position she can hold her head up.

October 8. The child is much improved. She swallows easily and takes food well. Facial paralysis remains very marked. The right knee jerk is not obtained, the left is present. Both Achilles reflexes are present. Movements of both legs are normal.

CASE 19.

Fred S., age, 5 years. Orange, N. J. Admitted, July 14, 1911. Discharged, July 30, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—One sister, 15 months old, admitted to this hospital at the same time as the boy, was paralyzed five days before him. Habitat: family lives in a separate house with a large yard. They have lived there for nine months. Last August, one year ago, there were two cases of paralysis, one an infant and one seventeen years old, occurring in different houses just across the street.

Past History.—Negative.

Present Illness.—The child vomited on July 19, the day the baby became sick. He was well for five days and then was taken sick with a high fever, 102° F., and complained of pain in his head. The next day the right side of the face was drawn up. There was no ptosis. The eye movements have been good. No trouble with swallowing. Can talk normally. Three days after the onset the left arm was limp for about twenty-four hours. Then the fever left and the condition has remained stationary. There has been no nasal discharge and no eruption. After the first twenty-four hours he seemed to have no pain. The bowels have been constipated during the illness. Appetite is good, no vomiting.

Physical Examination.—The patient is a bright active child who responds readily to questions. The head is well formed and held in normal position. *Eyes.*—External ocular movements are good, no ptosis. The left palpebral fissure is wider than the right. Pupils are equal and react to light and accommodation actively. The scleræ and conjunctivæ are clear. *Ears.*—No tenderness over the mastoids, no discharge. Hearing is acute. *Face.*—The right side is contracted and the left side lax and drooping. The mouth is drawn down partially to the right when in repose and moves well upward to the right on smiling. Both sides of the forehead can be wrinkled, but the wrinkles on the right go higher than on the left. On frowning, contraction is equal on both sides. He uses both eyes well. Masseters strong on both sides. *Mouth.*—The tongue can be protruded in the mid-line. Lips and mucous membrane are of good color. Teeth are good. The tongue has a slight white coat. Tonsils quite large on the right, easily visible on the left. They are not injected, their muscles move well. *Neck.*—Muscles are strong, no rigidity or tenderness. *Superficial lymph nodes.*—The right cervical, both axillary, and both inguinal glands are easily palpable, but not especially enlarged. *Thorax.*—Well formed; costal angle ninety degrees.

The chest moves easily with respiration. No rachitic rosary. Expansion good and equal. The thorax is everywhere resonant on percussion and clear on auscultation. *Heart.*—Negative. *Abdomen.*—Soft throughout; no tenderness or rigidity; moves with respiration. The muscles are strong. There are no masses. *Liver.*—Dullness extends from the fourth interspace to the costal margin in the right mammary line. Edge is not felt. *Spleen.*—The edge of the spleen is just felt on deep inspiration. *Extremities.*—No weakness of the arms. Grip a trifle stronger on the right than on the left. Brachial reflexes are active on both sides. Movements and strength of the legs are unimpaired. Patellar reflexes very active on both sides. Achilles reflex obtained on both sides. Plantar responses normal. *Sensorium.*—No hyperesthesia or muscular tenderness. *Temperature.*—99° F.

July 16. Blood count: leukocytes, 13,400; differential count of 200 cells: polymorphonuclears, 58.5 per cent.; small mononuclears, 25.5 per cent.; large mononuclears, 14.5 per cent.; eosinophiles, 0.5 per cent.; transitionals, 1 per cent.; red cells, normal in size and shape; no nucleated forms seen. General condition is good. The arms and legs move perfectly well. The abdominal muscles are normal. There is paralysis of muscles of expression on the left side of the face (seventh nerve); the masseter is normal. The tongue protrudes slightly to the right. The folds of the face are obliterated on the left, the right side of the mouth is drawn up. The right eye is partially closed by drawing up of cheek. Pupillary and eye reactions are normal. Hearing is apparently good and equal on both sides.

July 29. The tongue protrudes to the right but it can be put out to the left. Face: the right side of the mouth is drawn upwards, especially when he laughs. The asymmetry is not marked when face is at rest. The forehead wrinkles on both sides but most on the right. Arms: movements of both are strong and normal. Legs move strongly and normally. Knee jerks and Achilles reflexes are active on both sides. Residual paralysis of the left side of the face (seventh nerve). Patient is discharged.

October 7. The child returned for observation. He is in good condition but the mother reports that he is very nervous and timid. He is afraid to go anywhere alone. His face is a little better, but still shows some asymmetry on smiling.

CASE 20.

Jerry E. S., age, 3 years and 10 months. Lakewood, N. J. Admitted, October 8, 1911. Discharged, November 14, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a single house, clean and airy. They have no animals. The family have been at Jefferson, N. H., for three months, and the children have all been well. Three families lived in the house there, but there was no sickness in the house. The house was clean, there were no parasites, and no pet animals except one dog. They know of no cases of infantile paralysis in Lakewood or Jefferson.

Past History.—Negative except for a birth palsy of the left arm.

Present Illness.—On October 4, four days ago, the child said that he did not feel well. "He laid around" and would not play or eat. The next day he felt worse and lay on the couch all day and seemed inclined to sleep. That afternoon

his speech was less plain. The next day, Friday, he was kept in bed all day; speech was about the same, he could talk, but not distinctly. He seemed rather dull and restless, but there was no evidence of pain until yesterday, when he kicked and cried and seemed to be in pain. Two days ago his mother noticed that he could not swallow, and his mouth seemed to fill with phlegm. He has not eaten or drunk anything for three days. He has tried to swallow milk and water but it comes back. He vomited at the onset and on the second day. Bowels have moved with enema, urination has been normal. Face has seemed symmetrical. The child has had no convulsions. He had a cough and raised some phlegm, but has difficulty in getting rid of it. He has not sweat at all and has had no chill, but has been feverish for the last three nights. There has been no evidence of paralysis of arms or legs. His speech has been worse than it is today.

Physical Examination.—The patient is a well nourished small boy. He lies on his side with head slightly retracted and knees drawn up. He objects to being turned on his back, and struggles actively to regain the lateral position. His face is flushed and he lies in a soporific condition from which he is easily aroused by any manipulation which tends to place him on his back. *Eyes.*—The left pupil is a trifle wider than the right; both react normally. Ocular motions are normal. There is no strabismus. *Ears.*—Normal. *Nose.*—Normal. *Mouth.*—Tongue is heavily coated, and the mouth is filled with thick grey mucous, which collects in the pharynx and which the patient cannot get rid of. He evidently cannot swallow or change the position of the fluids in his mouth. Pharynx is reddened, tonsils are somewhat enlarged. *Neck.*—The neck is a little stiff to flexion. Muscles are weak and head lolls back when the trunk is raised forward. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are palpable but not enlarged. *Thorax.*—Well formed. Moves properly in respiration. *Lungs.*—Clear. *Heart.*—Normal. *Abdomen.*—Soft, not distended, no masses or tenderness. Liver and spleen not felt. *Extremities.*—Upper are normal and strong. Lower are strong and active. Knee jerks on both sides are, perhaps, slightly increased. *Temperature.*—101.4° F.

October 8. Spinal fluid: pressure not increased; cells, 18 per c.mm.; globulin, very slightly hazy; sugar, + (?). Blood count: leukocytes, 19,300; differential count of 200 cells: polymorphonuclears, 73 per cent.; lymphocytes, 18.6 per cent.; large mononuclears, 2.6 per cent.; transitionals, 4.6 per cent.; basophiles, 0; eosinophiles, 0; stimulation form, 0.6 per cent.

October 10. The child is somewhat brighter. There is no return of swallowing. He is receiving nasal feedings of milk and eggs, and salt solution by rectum. He usually regurgitates after the feedings and sometimes becomes quite blue. He has great difficulty in expelling the regurgitated food and the mucus from his mouth. When told to stick his tongue out he says, "I can't." When he tries he hardly gets it beyond his teeth, and it protrudes quite markedly to the right. No other paralysis is found.

October 12. Tongue paralysis is very definite, chiefly on the right side. Swallowing shows no improvement. The child is fed by nasal catheter. Speech has improved considerably since admission, but is still far from distinct. In general the child is somewhat brighter.

Spinal fluid: a few c.c. of clear watery fluid; no increase of pressure; cells, 90 per c.mm.; globulin, very slightly +; sugar, +.

October 14. The tongue protrudes definitely to the right. It is still impossible for him to swallow. Speech is not at all clear. The face is symmetrical. The pupils are equal. The neck resists flexion, which is painful. Movement of both legs is painful especially the right. He keeps the right leg flexed at the knee, and, when, any one handles it, resists any attempt to straighten the leg, this being very painful. Pressure over the calf and below the patient's leg is painful. The right knee jerk is not obtained (when the leg is held fairly flexed). Knee jerks and Achilles reflexes are active on the left. The movements are less painful.

October 20. The child is still being fed by stomach tube. He is not, however, losing much weight. For the last few days he has been getting more quiet, and takes very little interest in his surroundings, but he rouses when his mother comes. Yesterday his voice became much more weak and hoarse. He said his throat hurt him. A laryngoscopic examination was made but the vocal cords could not be seen. Today he breathes quietly, but has said very little and speaks in a very faint voice. His tongue deviates to the right. There are no other paralyses. The reflexes are normal.

Later in the day. Spinal fluid: clear, colorless; cells, 3 per c.mm.; mononuclears, +; sugar, +; globulin, slightly +.

October 21. Yesterday the patient caused surprise by swallowing a few cubic centimeters of water. Later in the evening he swallowed 20 c.c. of milk. This morning he took 150 c.c. of egg nog without difficulty.

October 30. The child swallows very well now, solid food better than liquids; in fact liquids still cause some trouble, but they have been swallowed much better in the last few days. His voice has been very weak and is still hoarse and weak but much better. He has complained greatly of pain, when being dressed or moved; but much of this seems to be fear, for today when his attention was diverted his legs were moved, and he moved them well himself and sat up straight without pillows and had no pain.

October 31. Spinal fluid: 5 c.c. of clear watery fluid; pressure not increased; cells, 3 per c.mm.; mononuclears, +; globulin, very slightly hazy (normal limits); sugar, +.

November 4. The patient walks better, but still with a swaying, shaky, almost ataxic gait. His legs are spread so that his feet are wide apart, and the right foot is usually held in outward rotation. The legs are moved and held rather stiffly. Both knee jerks are exaggerated, and both Achilles tendons give almost clonic response. There is a suggestion of ankle clonus but not a true clonus. The psychic condition is very interesting. The child is normally a rather quiet, silent, stubborn child. He is now distinctly nervously "on edge" and is ready at any moment to laugh or cry. Usually he ends by crying and without any particular reason. At times when some one is playing with him, he only answers by a series of nervous grunts, something between a giggle and a sob.

November 13. The child is in good general condition. He is still somewhat nervous and cries rather easily, but is quite different from what he was a fortnight ago. The child swallows all foods normally. His voice is loud but somewhat husky. The tongue still protrudes toward the right, and there is slight atrophy of the right side of the tongue. Both legs move actively and normally. Knee jerks and Achilles reflexes are exaggerated on the right, much exaggerated

on the left. Babinski and Kernig's signs are negative. There is a suggestion of ankle clonus on the left. The gait is much improved. He walks well and can run but still spreads his feet rather widely. His gait is perfectly steady, however. There is slight Romberg's sign. The child is discharged today.

CASE 21.

Helen N., age, 6 years. New York City. Admitted, September 17, 1911. Discharged, October 9, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. The family lives on West 57th Street. There are ten families in the house, one other on the floor on which the patient lives. The house is old and fairly clean and airy. There are many waterbugs and bedbugs, but very few flies and mosquitoes. There are, however, many fleas as the family has a pet dog and pups. There are no sick children in the neighborhood.

Past History.—The patient has always been a healthy child.

Present Illness.—Last Sunday, September 10, seven days ago, the patient was perfectly well and happy. On Monday evening she complained of nausea and vomited a little. From that time on she began to be dull, drowsy, and slept most of the time. She complained of headache and tired feeling. Wednesday evening she was a little irrational. A doctor was called, who said that the child had diphtheria and gave her antitoxin on Thursday morning. On Friday she did not recognize the family and had a vacant stare. There were no convulsions, but twitchings of the hands, feet, and eyelids. Another doctor saw her Friday and said she had meningitis. Temperature was then 105° F. On Saturday the patient was in a rather deep coma. Bowels have been constipated but moved yesterday with enema. The child has not spoken for the last two days.

Physical Examination.—The patient is a fairly nourished little girl who lies on her back, still, and apparently semicomatose. Occasionally the eyes roll under the half closed lids. She can be roused by handling. When the feet and hands are prodded, the legs or arms are drawn away sharply. She breathes regularly and by the normal mechanism, though perhaps a trifle less deeply than is normal. The face is rather flushed and has a slightly bluish tinge. *Eyes.*—The pupils are narrow, the right just a little smaller than the left. The eyes do not move quite coördinately. The conjunctivæ are injected, and there is a purulent secretion in the left eye. Eye grounds are apparently normal. The disc is sharply defined. The veins look a little full. *Nose.*—No excoriations or discharge. *Mouth.*—The teeth are in poor condition, some of the front ones broken. The buccal mucous membrane is reddened. The tongue is heavily coated. The pharynx is very red, the tonsils large. There is a thick mucopurulent mass in the pharynx with some yellowish white membrane-like shreds. *Superficial lymph nodes.*—Posterior cervical, axillary, and inguinal nodes are palpable. *Neck.*—Not rigid. *Thorax.*—Moves properly, though slightly in respiration. *Lungs.*—Clear. *Heart.*—Action is regular, not rapid, sounds not very loud. *Abdomen.*—Normal. *Extremities.*—Upper: apparently normal. Lower: power is apparently good. Both knee jerks are absent, both Achilles reflexes active. Babinski and Kernig's signs are absent on both sides. There is slight reaction to pain at the end of Kernig manipulation. *Surface.*—Clear. *Temperature.*—101° F.

September 17. Blood count: leukocytes, 23,200; differential count of 200

cells: polymorphonuclears, 76 per cent.; lymphocytes, 17 per cent.; large mononuclears, 3 per cent.; transitionals, 2.5 per cent.; eosinophiles, 1 per cent.; basophiles, 0; stimulation form, 0.5 per cent.

Spinal fluid: 25 c.c. of clear fluid; pressure 14 cm.; cells, 37 per c.mm.; mononuclears much increased; globulin, slight haziness, well within normal limits; sugar, +.

September 18. The child lies on her back in a drowsy condition. There are no rose spots and the spleen is not felt. There is no muscle stiffness of the neck, and no Kernig's sign.

P. M. There is slight but distinct weakness of the muscles closing the right eye.

September 19. This morning the patient is a great deal brighter. She looks at things about her a little more intelligently and she will slowly follow with her eyes an object that is moved in front of her face. She will answer simple questions. The ocular motions seem to be coördinate but slow. The pupils are equal and react sharply to light. The right palpebral fissure is a little wider than the left, and the patient is unable to close the eye. There is also very slight flattening of the right side of the face. No weakness of arms or legs is found. The knee jerks are still absent. The temperature which was 102.2° F. on the day of admission has been falling steadily for the past 36 hours and is now 99.2° F. The pulse rate has followed a parallel course. The von Pirquet reaction is negative. The neck is perhaps a trifle stiffer than yesterday. The patient complains of pain when her shoulders are lifted forward.

P. M. The patient is found wide eyed and crying. When questioned she says that she is better and wants to go home. Her mind is almost entirely clear, but she is a little slow in responding. The right knee jerk was obtained once or twice after repeated attempts, but not the left. No further paralysis is noted beyond that of the muscles supplied by the seventh nerve.

Spinal fluid: 22 c.c. of perfectly clear fluid; pressure slightly increased; cells, 44 per c.mm.; mononuclears much increased; globulin, very slightly +; sugar, +.

September 20. This morning the patient is still brighter. She lies quietly, however, preferably with knees drawn up, and does not like to be handled much. There is definite tenderness over the muscles on either side of the spine between the shoulders. There is no weakness in the arms or legs. The knee jerks are not obtained. Right facial paralysis is marked this morning. It is complete in the upper half with only weakness of the lower. The patient was taken from bed and put on the floor. She was a little unsteady in balance, but could stand and also walk.

September 21. Spinal fluid: 5 c.c. of clear limpid fluid; pressure not increased; cell count, 57 per c.mm.; globulin, very slight haziness; sugar, +.

September 25. This morning the child is bright and happy. During the last few days she has been inclined to be irritable. The paralysis of the right seventh nerve is receding a little. She cannot close the right eye. The knee jerks have returned on both sides. Achilles reflexes are as before. The child sits easily and also stands.

September 27. The patient is better. The knee jerks are both active. Paralysis of the upper half of the distribution of the seventh nerve is still marked. Spinal fluid: 25 c.c. of clear limpid fluid; pressure slightly increased; cells, 11 per c.mm.; globulin, very slight haziness; sugar, +.

October 9. No change in facial paralysis. The knee jerks are very markedly exaggerated, especially the right, which responds with almost a clonus. The Achilles jerks, too, are much exaggerated. There is no weakness in the extremities. The patient walks well and her general condition is good.

CASE 22.

Johanna W., age, 3½ months. New York City. Admitted, October 13, 1911. Died, October 15, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—This baby slept in the same bed with her sister, Margaret, who was taken sick on October 4 and became paralyzed on October 5. Margaret used to let the baby suck the nipple of her nursing bottle.

Past History.—The child is a nursing baby and has always been very strong.

Present Illness.—On Wednesday, October 11, two days ago, the mother noticed that the baby looked sick and feverish. The next day she was taken to a doctor who said that the child had a fever of 102° F., but did not pronounce it infantile paralysis. She has not vomited until today when she vomited in the examining room, nor has she sweat much. She has had no convulsions and has not seemed to have much pain. The main symptom has been sleepiness. Bowels moved with medicine; movements green.

Physical Examination.—The patient is a well nourished infant who seems drowsy. When handled she tries to cry, but cannot make any noise. She looks like a very sick baby. *Eyes.*—Pupils are equal. There is possibly a slight convergent strabismus. *Nose.*—The alæ nasi move slightly in respiration. *Mouth.*—The tongue is slightly coated with a whitish fur. Pharynx is reddened, tonsils somewhat large. *Head.*—Anterior fontanelle bulges a very little (diminished after lumbar puncture). *Neck.*—Patient cannot hold her head up. The posterior muscles are not stiff to flexion. *Superficial lymph nodes.*—The posterior cervical and occipital nodes are palpable. *Thorax.*—Well formed. It does not move properly in respiration. The whole thoracic cavity is drawn down with each inspiration. There is apparently complete intercostal paralysis. *Lungs.*—Clear. *Heart.*—Apparently normal. *Abdomen.*—Full, not distended. The urinary bladder reaches nearly to the umbilicus. Patient does not void. Abdominal respiration is marked. Liver and spleen are not felt. *Extremities.*—October 13. There is complete flaccid paralysis of both lower extremities this afternoon. Knee jerks and Achilles reflexes are absent on both sides. There is no Kernig's sign present and Kernig manipulation does not cause pain. There is apparently flaccid paralysis also of the right deltoid and upper arm. The left has tone. October 14. The left arm also seems to be flaccid this morning. *Surface.*—A few fine petechiæ are scattered over the surface.

Spinal fluid: 30 c.c. of very slightly opalescent fluid, pressure not increased; cells, 423; globulin, slightly +; sugar, +.

Blood count: leukocytes, 25,300; differential count of 200 cells: polymorphonuclears, 59 per cent.; transitionals, 13.5 per cent.; lymphocytes, 23.5 per cent.; basophiles, 0.5 per cent.; large mononuclears, 2.5 per cent.; eosinophiles, 0; stimulation form, 1 per cent.

The child is drooling at the mouth. Her face and lips are cyanotic. The respirations are short and shallow and the lungs clear. There is practically no movement of the ribs on the left side, although there may be slight retraction

on inspiration; on the right side the ribs move slightly. Abdomen moves with respiration but the diaphragm seems weak. At intervals there is a deep forced inspiration. The accessory muscles of respiration are called into play.

Respiration is irregular this afternoon, and at times is of a Cheyne-Stokes character, two or three deep forcible inspirations and then a pause in which one hears a few very shallow and weak respirations; then again two or three deep breaths. The strong expirations are associated with a forcible jerky upward movement of the diaphragm. Pressure on the abdomen and blocking of the action of the diaphragm causes great distress and difficulty with respiration. The child is very limp; there is apparently paralysis of the legs, intercostals, arms, and neck. She is very pale and slightly cyanotic. The accessory muscles of respiration (sternomastoids) are used, and occasionally the child throws her head back and her lower jaw forward with inspiration.

October 15. The child gradually grew weaker during the night and, without change in symptoms, died at 6:30 A. M.

CASE 23.

Helen K., age, 3 years and 10 months. Bergenfield, N. J. Admitted, August 19, 1911. Died, August 21, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives alone in a house in the country. The next house is twenty-five feet away. The house is new and clean. They have one pet dog which is healthy. No parasites. No possible contact of child or parents with anyone lame. The family has lived in the country for five years.

Past History.—The child was normal at birth, had chicken pox as a baby and measles last year. No scarlet fever or diphtheria. She has always had a weak stomach and vomits occasionally.

Present Illness.—Last Wednesday, August 16, three days ago, the child went to Rockaway. The day before she was perfectly well. She ate very little breakfast that morning before starting for the seashore, vomited while on the cars, but on arrival ate a big dinner. In the afternoon she went wading in the surf and had a chocolate soda. Then she began to feel sick and complained of a headache. She ate no supper. At 8 P. M. she vomited, and vomited three times that night. She was restless at night and very feverish. No especial tenderness, but rather irritable. The next morning she vomited again. The doctor thought she had "wind on the stomach." At this time she walked but was "weak on her legs." There was no change all day. Yesterday she was about the same. Her legs were weak, she could walk, but did not want to stand. She sat up last evening and fed herself at supper. The mother says her arms and legs "quivered" yesterday. At three o'clock this morning the mother put her on the water closet and she "collapsed." The mother then noticed that she could not move her arms. She has had a fair appetite, has vomited frequently, and the bowels have been constipated. No convulsions, no especial sweating. She has been very dull and sleepy for several days. Her mother thinks she passed no urine yesterday. She complained of headache at first and pain in the back, but not in the arms and legs. Two days ago her voice became very weak. It is more normal now.

Physical Examination.—The patient is a well built, apparently strong child.

Sensorium is clear, but she is distinctly dull and sleepy. The cheeks are somewhat flushed, lips and conjunctivæ of good color. The face is symmetrical. *Eyes.*—Pupils are equal, regular and react normally. Ocular movements are normal. No strabismus. *Ears.*—No discharge or tenderness. *Nose.*—No discharge or excoriation. *Mouth.*—The tongue protrudes straight and has a thick gray coat. Lips are dry, no herpes. The teeth are in fair condition. Both tonsils are large but not reddened, and there is no exudate. *Neck.*—The neck resists flexion and attempt to flex is painful. *Chest.*—Well formed and symmetrical. Costal angle just under ninety degrees. Respiration is almost wholly abdominal; chest moves very slightly with respiration. When the hand is put over the abdomen and pressure made to obstruct the diaphragm, there is no increase in thoracic respiration, and she complains that she cannot breathe and respiration gets more rapid. There is no marked rosary or Harrison's groove. *Lungs.*—Inspiration is short, clear throughout on auscultation and percussion. *Heart.*—No enlargement. Left border inside nipple line. Action is regular and rapid. Sounds are of good quality. A soft systolic murmur is heard over the precordium. *Abdomen.*—Level, soft, symmetrical, tympanitic, no masses or tenderness. Abdominal reflexes not obtained. No tache cérébrale. *Liver.*—Flatness extends from the sixth rib to the costal margin, edge not felt. *Spleen.*—Not felt. *Muscular system.*—Legs: movements of both legs are possible, no paralysis can be made out. There is possibly some weakness, but the child is so sick that it is hard to be certain of this. The Achilles reflex is present on both sides. The other reflexes are negative. The left quadriceps acts well. The attempt to elicit Kernig's sign causes pain and muscular spasm which prevents complete extension. Arms: the shoulder muscles are completely paralyzed. The child can flex and extend the forearms at the elbows but does so very weakly. Flexion and extension of the fingers and extension of the hands dorsally are possible, but all movements are weak. The child can sit up, but the neck muscles are weak and the head has a tendency to drop backwards or sideways. The back muscles seem strong. She sits up well. Sensation: there is pain in the back of the neck on flexion and on attempting Kernig's sign. No especial hyperesthesia noted. The voice is somewhat rasping and harsh, but strong.

August 19. Blood count: leukocytes, 10,000; differential count of 200 cells: polymorphonuclears, 61 per cent.; large mononuclears, 6.5 per cent.; transitionals, 5 per cent.; lymphocytes, 26.5 per cent.; eosinophiles, 0.5 per cent.; stimulation form, 0.5 per cent. Spinal fluid: 20 c.c. of absolutely clear, colorless, watery fluid; pressure, 180 mm.; cell count, 120 per c.mm.; sugar, +; globulin, very slight turbidity, normal; smear, practically all the cells are mononuclears and nearly all are lymphocytes.

August 20. The temperature is still elevated. The weakness of the hands seems more marked. Legs: there is definite weakness of the quadriceps on both sides. Knee jerks are both absent. Other movements are pretty strong. The Achilles reflex is not obtained on the right, and is slight on the left. The respiration is 42 to the minute; it is wholly abdominal and is very jerky. On pressure over the abdomen and upwards towards the diaphragm, respiration becomes more rapid and labored, and her face becomes quite flushed. No movement of the chest surely referable to intercostals was discovered. The child complains of pain in the back, but of no other tenderness.

August 21. Urine examination: yellow, turbid; reaction acid; Fehling's test for sugar negative; test for albumin with heat and acetic acid negative, with potassium ferrocyanide negative. No change in the child's condition yesterday. She was restless during the night but respiration remained the same until it suddenly changed at 7 o'clock this morning and the doctor was sent for. The child is lying on her back. The sensorium seems perfectly clear. The skin has a pale grayish color. The lips are dark red, rather cyanotic. The child is in a profuse sweat. The pupils are equal, regular, and react normally. Ocular movements are normal. The tongue protrudes straight. The temperature is normal. The child has difficulty in swallowing but takes some milk. She raises frothy mucus which she does not spit out, but holds between her lips and waits for it to be wiped off. Her respiration is short, jerky, and wholly abdominal. The upper part of the chest moves with respiration but is pulled down on inspiration. The abdominal respiration is normal. With inspiration the alæ nasi dilate, and there is a protrusion of the lower jaw. The sternomastoids and muscles of the neck stand out. Lungs: the right front is clear, respiration normal; left front, the respiration is very feeble; both backs are full of coarse moist rales. The heart is regular and normal. The abdomen is negative. The legs are as before, perhaps weaker. The arms are as before, movements of the hands weaker. The general condition is quite characteristic. She is perfectly clear mentally and speaks clearly but rather abruptly, telling exactly what she wants, "My arm hurts," "Turn me over," "Scratch my nostril." Apart from this, she desires to be let absolutely alone. "Don't touch my chest," "Leave me alone, doctor," she says. Her bowels have moved twice; after each movement the child is somewhat more cyanotic.

10 A. M. The child is very cyanotic, her respiration is irregular and jerky. She is moribund. The pulse is regular at 64; then it suddenly becomes more rapid, perhaps 100 (still regular) for a series of beats, then gradually drops down to about 64 again. Respiration becomes more feeble and suddenly ceases. The heart continues to beat forcibly and regularly for some minutes. Then it becomes more feeble and somewhat irregular, though in general the arrhythmia is of the type with a series of beats at regular rhythm, changing to a series of regular beats at another rate. The heart beats become more infrequent, and the heart stops beating five and a half minutes after respiration stopped.

CASE 24.

Alfred K., age, 15 months. New York City. Admitted, October 14, 1911. Died, October 17, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in bad surroundings. The house is an old wooden structure with a bakery in front and living rooms in the rear. The bedrooms are dark. There are flies, mosquitoes, and all varieties of vermin. There have been no acute infections in the house lately. There have been no cases of poliomyelitis in the immediate neighborhood, but there was one case two blocks away "some time ago."

Past History.—The patient has always been a healthy child with the exception of some slight gastro-intestinal trouble.

Present Illness.—The onset was rather sudden on Monday night, October 9, five days ago. The child was restless, could not sleep, cried a great deal, and seemed to have pain in the head. There was no spontaneous vomiting, but the child was

given ipecac and then vomited a little frothy mucus. On the next day he had a fever. Three days ago he had a slight convulsion, and on the following night had short general convulsions involving the whole body with the exception of the lower extremities. He has had muscular twitchings and a coarse tremor of the hands. There has been no nasal discharge. Sphincters have not been affected. Two days ago paralysis of both legs was noticed and at that time all reflexes were absent. Yesterday there was beginning weakness of the right arm. There has been no respiratory involvement. Temperature, pulse, and respiration have been as follows: October 10, temperature 102.6° F., pulse 140, respiration 60. October 11, temperature 102.6° F., respiration 48. October 12, temperature 101° F., pulse 130, respiration 36. October 13, temperature 100.2° F., pulse 120. October 14, temperature 98.6° F. Child has been very constipated, urination has been normal.

Physical Examination.—The child is a well developed, strong baby, lying quietly on his back. The eyes are open and the child is not asleep, but he seems languid and takes no interest in anything. The head is square and the cranium bulging. Anterior fontanelle is not closed. The face is symmetrical. *Eyes.*—Pupils are equal, regular, and react normally. Ocular movements are normal. Eyelids close normally. *Ears.*—No discharge or tenderness. *Nose.*—No discharge or excoriation. *Mouth.*—The tongue has a thick gray coat. There is a distinct groove in the median line. The lips are dry. Both tonsils are enlarged and boggy; no exudate and no especial redness. *Superficial lymph nodes.*—There are small glands in the neck, axillæ, and groins. *Neck.*—The child resists flexion, it evidently causes pain. *Chest.*—Well formed, costal angle ninety degrees, moves normally with respiration, no rosary or Harrison's groove. *Lungs.*—Clear throughout. *Heart.*—Normal in size, sounds clear. *Abdomen.*—Full, very soft, tympanitic, no masses or tenderness. Abdominal reflexes are barely obtained. There is no tache cérébrale. The abdominal muscles lack tone and seem very weak. There is no contraction when the child sits up. *Liver.*—Flatness extends from the sixth rib to the costal margin. Edge is just felt. *Spleen.*—Not felt. *Muscular system.*—Movements of the arms seem strong. Legs: both knee jerks, both Achilles reflexes, and both Kernig's signs are absent. There is no resistance to the Kernig manipulation on the right, and on the left only slight tightening of the external hamstring as the leg is extended (muscles all paralyzed), but extension is quite painful. There is no tenderness on pressure over the legs. Back: when the child is made to sit up the body falls forward over the legs, bending at the hips. There is paralysis of the gluteals, erector spinæ, and neck muscles. The head cannot be held up but drops loosely forward, backward, or to one side. Cremasteric reflexes are active. *Surface.*—The skin is rather dry but not hot. There is no rash. The lips are slightly pale.

October 17. Spinal fluid: 6 c.c. of clear, colorless, watery fluid, pressure low; cell count, 114 per c.mm.; mononuclears much increased; globulin, very slightly +; sugar, +.

The child does not look so well. He appears weaker and his respiration is rapid. The temperature has not risen. The child has some difficulty in swallowing, but can still take food. The tongue is apparently straight, but he will not stick it out. The chest moves well with respiration. The accessory muscles take some part in respiration. The abdomen moves paradoxically. There is distinct

retraction on inspiration, paralysis or weakness of the diaphragm associated with the weakness of the abdominal muscles noted before. On auscultation both sides of the chest are full of coarse moist rales, the fronts more so than the backs; there is no consolidation. The legs are paralyzed. The arms and face are unaffected. The child does not cry at all loudly (laryngeal involvement?).

11:30 A. M. The child has changed remarkably in the last hour. He is quite cyanotic and the respiration is short and labored. Breathing has become worse and the heart irregular (Luciani periods) until respiration practically stopped. Artificial respiration was then begun and camphorated ether injected. In a few minutes the heart was regular and rapid, and respiration fairly regular and deeper than before. His color was much better, and child could breathe alone. The intercostal muscles appear to be weakening now. With each respiration the lower ribs flare, but there is little or no movement of the upper ribs. The diaphragm is not descending. There is increasing activity of the accessory muscles of respiration in the neck. They are acting strongly, and with each inspiration there is a depression and protrusion of the lower jaw. The irregularity of the heart seems to bear some relation to cyanosis, increasing as the color gets worse. The child is unconscious. The skin has been gray and cyanotic since this attack began. It is quite edematous and marks of the stethoscope over the chest and also over the shins persist for a long time. During the period of apnea which occurred suddenly and in which the child appeared to be dying, the heart action assumed a curious irregularity, but one which was similar to that which we have seen in several other cases of respiratory failure. The heart would beat regularly at a rate of about 84 for a period of 10 seconds and then at a rate of 36 for 6 or 7 beats. This alternation of rapid and slow series of beats occurred over a considerable period of time, probably a minute and a half or two minutes. During the latter part of this time, artificial respiration was being carried on, and the heart action then became regular and rather rapid. When the patient began again to breathe spontaneously, the heart rate became rather slower and continued to be regular. After about 10 or 15 minutes the child again had respiratory failure. This time, however, the heart did not show the rhythmical changes in rate, but gradually became slower and weaker with slight sinus arrhythmia from time to time. The heart continued to beat, giving the normal sounds, at a fairly regular rhythm for three minutes after the breathing finally stopped, at least nothing could be heard after three minutes, except possibly the vaguest sound in the region of the second right interspace. Electrocardiographic records, however, show that an action current was formed for 24 minutes after breathing stopped and 21 minutes after the sounds had become inaudible.

CASE 25.

Jesse L., age, 5½ years. New York City. Admitted, July 20, 1911. Died, July 20, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a fifteen family house and knows of no sickness in the same house. The child and the parents do not remember ever having come in contact with anyone who is lame or paralyzed. The child has been in New York since last summer when he went for a few days to the country. There are no animals in the house. The building is old, but fairly clean and airy.

Past History.—The child has always been perfectly well.

Present Illness.—On Wednesday, July 12, eight days ago, the mother noticed that the child was feverish. She gave him citrate of magnesia, and the next morning he appeared to be well. On the following three days, Thursday, Friday, and Saturday, the child was up and played normally. Saturday night, however, five days ago, the child complained of pain in the back and was very restless and tired. He slept poorly and the next morning complained of severe pain in the head and back. He was given castor oil and vomited. Dr. Taylor saw the child in the evening. At this time he had pain and a stiff neck and his temperature was 102° F. Note by Dr. F. L. Taylor, who has had the case under his care since July 16: "The child has been ill for three days—apparently some digestive disturbance and coryza. Was given castor oil by mother. Last night complained of intense frontal and occipital headache; pain in the back of the neck and upper dorsal spine with retraction of head. Tendon reflexes were perhaps slightly exaggerated. There was twitching of the individual muscles of the extremities as the patient lay in bed. Temperature 102.2° F. (rectal); pulse 150. Has Kernig's sign and no Babinski reflex. Respiration 50 to 60 and shallow. Heart, lungs, and abdomen negative. Leukocyte count, 7,000; polynuclears, 81 per cent. Monday morning, July 17, condition unchanged. Lumbar puncture: 15 c.c. of crystal clear cerebrospinal fluid obtained under some pressure (*i. e.* came out in a spurt). Differential count shows polynuclears, 71 per cent.; lymphocytes, 21 + per cent.; endothelial cells, 2 + per cent.; incubation negative. Tuesday: reflexes obtained; tendency to somnolence; takes nourishment well. Temperature, pulse, and respiration about the same. Wednesday afternoon: respiration 36, temperature 101° F., pulse 120, quite somnolent, very weak. Urine: specific gravity 1,009, slightly acid, clear, heavy deposit of phosphates, no albumin, no sugar. Cell count: leukocytes, 3,000; polynuclears, 58 per cent.; small lymphocytes, 28 per cent.; mononuclears, 14 per cent. During the night the child had great difficulty in breathing, partial paralysis of the muscles of respiration, left arm, and left leg." The mother says that she first noticed weakness of the legs on July 18, two days ago. There has been no vomiting other than at the onset except yesterday when the child vomited once; no undue sweating. The mind has been clear.

Physical Examination.—The patient is a well built little boy, lying on his back. He is perfectly conscious and aware of all that goes on around him, but talks with difficulty in a very weak voice. Respiration is very labored and rapid, the alæ nasi dilating with inspiration, and with each inspiration there is a marked contraction of the sternomastoids on both sides. Associated with this is an extension of the neck and a protrusion of the jaw. The chest itself does not move at all with respiration. The abdomen moves in a normal manner, but the excursion of the abdominal wall is limited and weak. The skin is clear and white. Mucous membranes are pale. There is no discharge from the nose. The lips are covered with dry crusts. *Eyes.*—Movements of the eyeballs and pupillary reactions are normal. *Ears.*—Hearing is apparently normal. There is no discharge and no tenderness over the mastoids. *Mouth.*—The tongue is coated and protrudes straight. The teeth are in good condition. The tonsils are enlarged and ragged; there is no exudate. No herpes. *Superficial lymph nodes.*—There are small glands in the neck, axillæ, and groins. *Chest.*—Well formed, costal

angle about ninety degrees. The chest does not move with respiration. *Heart.*—Normal. There is a systolic murmur all over the precordium, most marked at the pulmonary area. *Lungs.*—Both fronts are normal on auscultation and percussion, but the breath sounds are very feeble. As far as can be ascertained, the backs are also normal, but turning the child over disturbs him greatly. *Abdomen.*—Level, soft, tympanitic, no masses or tenderness. Abdominal wall moves normally with respiration, but to a limited extent. The abdomen is symmetrical. *Liver.*—Flatness extends from the sixth rib to the costal margin. The edge is not felt. *Spleen.*—Not felt. *Muscular system.*—Legs: the Achilles reflex is present on the right side, the other reflexes are negative. Movements of the legs: it is difficult to ascertain how far the legs are paralyzed and how far lack of movement is due to weakness alone. His mother says that he can move the right leg, but no movements were seen. The left leg is very weak and flaccid, apparently completely paralyzed. Arms: the hands, wrists, and forearms can be moved on both sides. The muscles of the shoulders are apparently paralyzed, for the child seems unable to move his upper arms. Face: the face is not quite symmetrical. There is slight smoothing out of the right side of the face. The folds on the left side are somewhat more marked than on the right. This becomes more definite when the child talks or smiles.

July 20. 3:30 P. M. The pulse is irregular. At the apex a series of beats is heard at a rate of about 100; then the rate suddenly changes to 120 to 140, and a series of regular beats occurs at this rate. The rhythm of both rates is regular. Each rate may continue for three to four beats or perhaps twenty or more beats, then there is a quick change to either a slower or faster rate. The fast rate is not so much as twice that of the slow rate. No abortive beats and no compensatory pauses are heard. Between the most rapid rate and the slowest, other variations in rate seem to occur. The arrhythmia reminds one of rapidly recurring attacks of paroxysmal tachycardia.

Later. After admission the child's condition became gradually worse. He could at first speak a few words, though articulation was difficult. His lips moved and one could see that he was trying to talk, and after a few attempts he managed to utter a few words, such as "a drink of water"; the words were clear and distinct. After the first few hours he became unconscious. Movements of the limbs were few, presumably from weakness. Respiration was very labored and seemed to involve a voluntary muscular effort. The chest wall did not move. There was paralysis of the intercostal muscles. The muscles coming into play most actively were the sternomastoids and the platysma on both sides. The scaleni appeared not to act. On admission, the diaphragm was acting normally, but weakly. The diaphragm became weaker and weaker, *i. e.*, the movement of the abdominal wall became less and less, and towards the end it was scarcely noticeable. As death approached, respiration continued to be very rapid, 40 to 50, was even more short and gasping than before, and seemed to be carried on almost wholly by the sternomastoids and other neck muscles. The heart meanwhile continued to act strongly, showing constantly the type of arrhythmia noted above, an irregular hurrying and then slowing, simulating more a rapidly recurrent paroxysmal tachycardia, than an auricular fibrillation with pulse intervals of constantly varying lengths. The strong action of the heart up to the end was in marked contrast to the constantly failing respiration. The child took no

nourishment, only sips of water. Whether or not he could swallow could not be determined. He refused even on admission to take more than just enough fluid at one time to moisten his lips, which were very parched. Respiration grew weaker, the child became unconscious and at 7 P. M. died, of respiratory failure. The child was extremely pale on admission. Cyanosis was not especially marked until the very end, and even then it was no more marked than one usually sees it just at death. It was at no time a prominent sign.

CASE 26.

Roger P., age, 4 years. Hawthorne, N. Y. Admitted, September 20, 1911. Died, September 23, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—The father and mother are living and well. There are two other boys in the family, aged two and nine years. Both have been perfectly well. All three children had measles last May. The two year old boy was slightly indisposed seven days ago. He was somewhat irritable and had little appetite but no vomiting or diarrhea. The family live in a small village in a house with grounds. The air and light are excellent. There are few mosquitoes, no roaches or other bugs, but large numbers of flies. The children have played about with other children in the neighborhood. T. R., who now has poliomyelitis in the Hospital, lives in the same village, but the patient had not been in the habit of playing with him. There are no other cases in the neighborhood.

Past History.—The patient has always been a strong healthy child.

Present Illness.—On Saturday, September 16, four days ago, the child suddenly complained of pain in the right leg. On trying to run about, the leg seemed somewhat weak. At that time he also had slight fever and very little appetite. He was kept in bed most of the time but on Monday afternoon, was up and able to walk about a little without assistance. He has not walked since, however. It has been noticed that his left arm has been very weak and also his back. The voice has seemed somewhat thin. He has not vomited and has had no convulsions. He has been rather drowsy, complained of headache and pain in the stomach, and has been constipated since Saturday. No retraction or rigidity of the neck has been noticed. On the evening of September 18, and yesterday morning, respiration was rapid and shallow, but it did not appear to be so last night. He has not urinated since this morning.

Physical Examination.—The child lies quietly on his back. He seems drowsy and does not respond readily to questions. The expression is rather dull. There is no evident distress. Color is good, skin hot and moist. *Head.*—The head is well formed; no frontal bosses. *Face.*—The right side of the face is slightly relaxed, and the mouth is drawn to the left on smiling. *Eyes.*—Pupils are equal, not dilated, react actively to light. There is no ptosis. External ocular movements are good. Scleræ and conjunctivæ are clear. *Ears.*—No discharge or tenderness. *Mouth.*—Lips and mucous membranes are of good color. The tongue has a moderately moist white coat. Pharynx is not injected. Tonsils are not large; no exudate. *Neck.*—The head falls back on sitting up, so that there is evidently paralysis of all the anterior muscles. There is no retraction, the child resists bending forward because of pain. *Superficial lymph nodes.*—Not enlarged. *Chest.*—Well formed and symmetrical. Expiration is almost imperceptible. Breathing is purely diaphragmatic. The lower ribs do not move on

inspiration. The upper portion of the thorax is pulled up slightly on inspiration, although there is no marked action of the accessory muscles. The diaphragm descends well but seems a little weak. There is no pulling in of the ribs at the attachment. Respiration is shallow with an occasional, deep, sighing inspiration. Lungs are clear on percussion and auscultation. *Abdomen.*—The abdomen moves normally with respiration. The muscles seem soft and relaxed. There is no bulging in the flanks, no masses or tenderness. Superficial reflexes are absent on both sides. *Extremities.*—Upper: the grip of both hands is weak. There is no wrist drop. The fingers can be extended and flexed. Movements of the forearm are good. There is paralysis of the deltoid on both sides. Reflexes are absent. Lower: there is complete flaccid paralysis of the left leg. The toes of the right foot can be slightly flexed. Right leg: there is paralysis of the quadriceps, iliopsoas, and extensors of the foot, and weakness of the posterior muscles of the right lower leg. The toes can be fairly well flexed. The right gluteus and the hamstrings are active. All reflexes are absent on both sides. An attempt to elicit the Kernig's sign causes pain, but no muscle spasm.

September 20. Blood count: leukocytes, 12,400; differential count of 200 cells: polymorphonuclears, 53 per cent.; transitionals, 12 per cent.; lymphocytes, 24 per cent.; basophiles, 0; large mononuclears, 9.5 per cent.; eosinophiles, 0; stimulation form, 1.5 per cent. Spinal fluid: 22 c.c. of clear fluid; pressure slightly increased; cells, 55 per c.mm.; almost all mononuclears; globulin, very slightly +; sugar, +.

Later, near midnight, the patient could be seen just beginning to use the accessory muscles in the neck, and the alæ nasi were also working.

September 21. The child slept fairly well. This morning the costal muscles seem entirely paralyzed, and the accessory muscles in the neck are working much more obviously. The diaphragm is acting but not strongly. The lungs are clear. There is localized sweating of the upper lip. Later in the morning: the respiratory embarrassment is much more marked. The right side of the abdomen moves more than the left with inspiration, as though the left side of the diaphragm were not working. The child is drowsy but is easily aroused, and then is a little irritable. He can move the forearms and hands but not the shoulders. The pupils are equal and contracted.

In the afternoon the diaphragm seems to gain strength and the abdomen moves evenly and with a somewhat greater excursion. The rate of respiration is rather low, considering the amount of respiratory embarrassment. The breath sounds are not heard below the junction of the lower and middle third of the axilla. There are no rales. Towards evening the heart rate increased and a sinus arrhythmia was noted. There was some difficulty in swallowing noted, but this seemed at first due as much to increase of respiratory embarrassment as to actual failure of the muscles of swallowing. Later, however, quite definite failure of deglutition was noted. The child throws his head from side to side at times and cries weakly, seemingly trying to escape from some oppression. He cannot articulate clearly. The eyes are bright, but often he dozes, and the eyeballs roll up beneath the incompletely closed lids.

September 22. The patient slept fairly well. This morning he is breathing with about the same degree of embarrassment as yesterday evening, perhaps a trifle more easily. The rate of inspiration varied between 22 and 30 during the

night. The temperature is falling by a fairly steep curve. The patient's color is good. He seems to be getting enough air. This morning he is rather more drowsy than he has been. He cannot articulate properly, but he swallows much better than yesterday evening. Occasionally he moves his head vigorously from side to side. The arms are moved more extensively than yesterday. The abdomen moves about as much in respiration as yesterday, perhaps the right side a little more than the left.

P. M. The patient has gone on well through the day. He breathes a trifle more easily this evening. The abdomen certainly makes a slightly greater excursion on inspiration than it did this morning. The child takes his fluids much better this evening. This morning he had much difficulty in swallowing. He is more willing to try to speak and succeeded in articulating and phonating the words "good-night" weakly but distinctly.

September 23. This morning about 5:30 the patient began to weaken markedly. His color is not so good; there is a faint tinge of cyanosis on the lips and duskiness about the eyes. He is pale. The breathing is much shallower than last night. The heart action is fairly strong. From time to time there is definite sinus arrhythmia. The child's mind is quite clear and he can speak. He knows perfectly well what he wants and does not want. The left side of the diaphragm evidently does not descend at all, for that side of the abdominal wall remains motionless. From time to time faint, blotchy, localized flushings appear on the face and fade again. At 10:35 A. M. the first heart sound has become rather tapping and sharp in quality. The action is somewhat slower and also more constantly irregular, though the arrhythmia is very slight.

P. M. The patient has been breathing less and less deeply through the day. Cyanosis of the finger tips has been gradually developing and a slightly more dusky appearance of the face and lips. The left side of the diaphragm is apparently not acting at all. The abdomen is becoming distinctly more scaphoid, and the tissues are losing their elasticity. The child is in a rather deep coma, from which he can be roused, however, by persistent prodding. He swallows with difficulty. About two hours before death the child suddenly seemed to come out of his coma for a few moments and looked about him with perfectly clear eyes and apparently recognized his parents. This transient return to consciousness was of extremely short duration, and he rapidly lapsed again into coma. Following immediately upon this, the child's breathing became suddenly worse and in a few moments stopped entirely. He looked as though he had died. During the period of respiratory pause which lasted, with the exception of one or two shallow gasps, for nearly two minutes, the heart continued to beat, at first regularly as before, but after a few seconds with a noticeable irregularity. This soon took the form of alternating series of regular rapid beats and regular slow beats. The sounds conveyed the impression that tracings from the heart taken at the time would have shown a typical curve of Luciani periods. At the end of the long respiratory pause, the patient suddenly began to gasp more deeply and in a few breaths had resumed his previous respiratory rhythm. This continued for nearly two hours longer and then stopped suddenly as it had in the first instance. Electrocardiographic records were taken at short intervals throughout the last eight or nine hours of life.

CASE 27.

George G., age, 9½ years. Brooklyn, N. Y. Admitted, August 30, 1911. Died, September 1, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in a double house, which is quite new and is clean and airy. No parasites are found in the house. There is no history of any contact of parents or child with any lame or paralyzed person.

Past History.—The child has always been healthy.

Present Illness.—The present illness began about a week ago. The child felt poorly, was given physic, and soon appeared perfectly well again. No definite symptoms were noted until Sunday, August 27, three days ago, when he complained of stiff neck. He vomited once the same day after a meal, but has not vomited since. He has had no convulsions, and the mother has noticed no profuse sweating. At the onset he had headache and indefinite pain in both thighs, lasting a few hours, but besides this has had no especial pain. Throughout the illness his mind has always been clear. He eats well, but his bowels are constipated. He has no trouble with voiding, no cough, or sore throat. He has been kept in bed, and his illness was considered to have been cerebrospinal meningitis. There was no history of paralysis until this morning (11:00 A. M.) when he had lost knee jerks, and the muscles of his legs were flaccid and motionless. Since then the left arm has become involved (noted at 4:00 P. M.). This morning his respiration was entirely abdominal and his thorax was motionless. Expiratory grunt was also noted. He has not been drowsy and not especially restless.

Physical Examination.—The patient is a rather slim, well built boy. He lies on his back with his eyes open and is evidently quite alert, noticing all that goes on. He responds to questions brightly but with a very weak voice. Respiration is rapid, rather jerky, and labored. The alæ nasi dilate with respiration and the muscles of the neck, especially the sternomastoids, take an active part in respiration. The chest itself is quite motionless. Respiration is wholly of the abdominal type, but the impression is given that the diaphragm is not acting very strongly. There is slight protrusion of the lower jaw with inspiration. The face is symmetrical. The skin is rather dark in color; no rash. *Eyes.*—The pupils are equal, regular, and react rapidly to light. Ocular movements are normal. Eye slits are equal. *Ears.*—No discharge or tenderness. *Nose.*—No discharge or excoriation. *Mouth.*—The tongue protrudes straight and has a thick gray coat. The tonsils are not enlarged. The pharynx is negative. The child can swallow milk well. *Neck.*—There is stiffness of the neck and resistance to flexion, but no retraction. *Superficial lymph nodes.*—The glands in the neck, axillæ, and groins are just palpable. *Chest.*—Well formed and symmetrical. *Lungs.*—Clear throughout on ausculation and percussion. *Heart.*—Not enlarged, action regular and rapid. Sounds are of good quality. A soft systolic murmur is heard all over the precordium, most marked in the pulmonary area. *Abdomen.*—Slightly distended, soft, tympanitic. No masses or tenderness. Abdominal reflexes and cremasteric reflexes are not obtained. *Muscular system.*—Legs: knee jerk and Achilles reflex are absent on both sides. Babinski reflex is negative on both sides and also Kernig's manipulation, which is slightly painful. Movements: no movements are obtained in either leg except flexion of the toes. Sensation: the

touch of a camel's hair brush is easily felt over both feet and lower legs. Arms: there is apparently a paralysis of both upper arms. The child can flex and extend the fingers weakly and can flex both elbows. Abdominal muscles: there seems to be some tone to the abdominal muscles. The neck and back muscles were not tested. The boy is so sick that an accurate localization of the paralysis is impossible.

August 30. Blood count: leukocytes, 16,700; differential count of 200 cells: polymorphonuclears, 75 per cent.; lymphocytes, 15 per cent.; large mononuclears, 0.5 per cent.; transitionals, 9.5 per cent.

August 31. The boy's condition is about the same. He is bright and alert and appears to be making a good fight. Each breath he takes comes with obvious effort. Except for breathing, however, he makes no effort to move. When he speaks it is in a low voice, and he only speaks in order to answer questions or to make known his requests. He says respiration is easier than last night. He asks to have his nightgown loose about his neck, to be turned over, etc. Physical examination shows nothing new. No rales in the lungs. The respiration is abdominal, and the movement of the abdomen seems weaker than last night. The upper part of the chest moves with respiration, but this movement seems to be due to the pull of the neck muscles. The arms seem a little weaker.

Afternoon. The excursion of the abdomen in respiration is very slight. Later in the evening there seemed to be a distinct improvement in the patient's condition. He answered questions more fully, moved his head more, and in general did not display the extreme economy of muscular effort seen earlier in the day. The breathing was distinctly less shallow. Abdominal excursion was fair. Urine examination: clear, amber; specific gravity 1,017; reaction acid; Fehling's sugar test negative; albumen test with heat and acetic acid negative; sediment, heavy, yellow, granular precipitate.

September 1. The patient dozed for short periods during the night. At 6 o'clock, breathing became worse. The patient was cyanosed, especially about the lips. The effort to expand the chest was great, and spent chiefly through the sternocleidomastoids, the muscles of the floor of the mouth, and the hyoids. The pull on the inner end of the clavicles was such that a partial anterosuperior dislocation of the sternoclavicular articulation resulted with each inspiration. Slight protrusion of the epigastric region indicated weak diaphragmatic action. This continued practically to the end. About one half to three quarters of an hour before death the heart action, which had been regular and moderately strong, began to show irregularity. At first this seemed to be an occasional sinus arrhythmia. A little later, however, alternating stretches of slower regular, and more rapid regular rates were observed. Sometimes the rapid period was entered by a gradually and evenly increasing rate. The slow rate was usually approached more abruptly by a few, irregular, slower beats. There was no change in the quality of the sounds. Notwithstanding the respiratory difficulty and the failing heart, the lungs remained clear of adventitious sounds until a few minutes before death, when some scattered rales appeared in the lower left axilla. The patient remained semi-conscious almost until the last, although a somewhat delirious muttering was present for two hours or more before death. There was a slight inequality of the pupils, the left being a little larger, during the last hour or more. The heart continued to beat very weakly and regularly for a minute or two after respiration ceased. The sounds could be heard over the base but not at the apex.

CASE 28.

Belle T., 8 months old. New York City. Admitted, August 21, 1911. Died, August 23, 1911. *Diagnosis.*—Acute poliomyelitis.

Family History.—The mother and father are living and well. There is one other child in the family, who subsequently also developed poliomyelitis and died.

Past History.—The child is one of twins. She has always been healthy except for erysipelas at the age of three months.

Present Illness.—On Friday, August 18, the mother noticed that the child was feverish. The doctor was called on Saturday morning. At this time, the child had a temperature of 103° F., pulse 120, and was very nervous. The throat was red and inflamed, and there was a slight patch on the right tonsil. In the evening the throat was clearer, but the temperature remained at 103° F., the breathing became more rapid, and a few rales were heard. The doctor said it looked like pneumonia. Later in the evening the temperature fell to 101° F., pulse 110. August 20, the child appeared brighter and not so nervous, but she was not so lively as usual. She slept well that night. Next morning, she was again seen by a physician who noticed twitching of the face and a peculiar movement of the toes of the left foot, almost rhythmical in character. About this time weakness was also noted in the left arm and leg. At noon the same day, August 21, Dr. Francis Huber made a diagnosis of poliomyelitis. During her illness there has been no vomiting and the bowels have been normal.

Physical Examination.—The patient is a well nourished infant who lies quietly and is rather somnolent. She frets a little if handled much, but lapses rapidly into the sleepy condition. The expression is a drowsy one, and not alert. *Eyes.*—There is a slight but definite internal strabismus. The pupils are equal and react normally to light and accommodation. *Ears.*—Normal. *Mouth.*—The buccal mucous membrane is clean. The pharynx is a trifle reddened. The tonsils are a little enlarged. *Head.*—The anterior fontanelle is open widely; no bulging or pulsation. *Neck.*—Not stiff. No power in the neck muscles is apparent; the head drops when the baby is sitting up. *Superficial lymph nodes.*—The cervical nodes are palpable. *Thorax.*—Well formed, moves very little in respiration. *Lungs.*—Apparently normal. *Heart.*—Apparently normal. *Abdomen.*—Rather prominent, moves properly and rather widely in respiration. It is soft, there are no masses, but palpation of the abdomen seems to produce discomfort. *Extremities.*—Upper right: no definite weakness is found. Left: there is definite loss of power in the shoulder muscles, probably in the biceps also. Flexors and extensors of the hand, and extensor of the forearm are weakened but not paralyzed. Lower right: there seems to be complete weakness of the thigh muscles. The flexors and extensors of the foot are still intact. Left: practically the same condition exists on the left though not quite so marked. The reflexes are absent on both sides.

August 21. This evening a few fine dry rales are heard along the inner border of the left scapula. Respirations are very rapid and short, but the diaphragm is working well. Blood count: leukocytes, 23,900; differential count of 200 cells: polymorphonuclears, 25 per cent.; large mononuclears, 2 per cent.; lymphocytes, 57 per cent.; transitionals, 25 per cent.; eosinophiles, 1 per cent.

August 22. Urine examination: pale yellow, clear; reaction acid; Fehling's test for sugar negative; test for albumen with potassium ferrocyanide negative; slight sediment.

August 23. Last evening the breathing, which had been extremely rapid since admission, was not so deep, and the alæ nasi were moving. They had not been active previously, even with the high respiratory rate. This morning the respiratory motions are much more short and jerky. The floor of the mouth and glottis are drawn down forcibly with each inspiration. There is considerable power in the dorsal flexors of the left foot. The right is much weaker. The knee jerks are both absent, likewise Achilles reflexes. The child is drowsy and apathetic. The heart action is regular and strong, 174 per minute. There are a few rather moist rales on the left lung. The abdomen seems to move more on the left side than on the right, and the viscera appear to move downward and toward the right with each contraction of the diaphragm, as though the left half of the muscle were acting more strongly than the right. It is almost impossible to determine whether the abdominal muscles are paralyzed. There seems to be a very faint reflex on the left, none on the right. During the morning the child's condition gradually becomes worse. From time to time beads of perspiration appear on the lips and forehead. The accessory muscles of respiration are brought more and more actively into play. There is a striking collapse of all the tissues. The rotundity of outline has gone almost visibly from the costal margins, the abdomen becoming more and more scaphoid and the tissues looking faded and inelastic. The motion of the left side of the abdomen is very marked, the right side is practically motionless. From time to time the tongue is protruded, and the lips are opened and shut as though to moisten the mouth. Frothy saliva covers the tongue and lips. There is cyanosis of the tongue, but the lips are marked rather by pallor than by blueness. The whole face is pale. Heart rate, 192 to the minute at 1:20 P. M., action strong and regular. The rales are still present over the left anterior axillary region. The breath sounds are somewhat better heard over the lower left chest than the lower right. The pupils are equal and remain strongly contracted. They react, however, to light. During the afternoon the respiration becomes constantly weaker. The left half of the diaphragm continues to act but the respirations are very shallow and jerky. The child dies suddenly at about 5 P. M.

CASE 29.

Frances K., age, 21 months. Perth Amboy, N. J. Admitted, September 11, 1911. Died, September 16, 1911. *Diagnosis.*—Acute poliomyelitis; bronchopneumonia.

Family History.—Negative.

Past History.—The child has always been healthy except for an attack of bronchitis last year.

Present Illness.—On Wednesday, September 6, the patient was perfectly well. On Thursday she had a slight fever, was restless, and evidently had pain when handled. The restlessness continued on Friday with loss of appetite and alternating constipation and diarrhea. On Saturday the doctor noticed paralysis of the right arm and slight weakness of the neck. There was an increase of paralysis of the neck on Sunday and the child vomited once. Today there is possibly some weakness of the left arm also. The child has had no convulsions. No urinary symptoms.

Physical Examination.—The patient is a well nourished little girl, who lies

quietly without moving and is very pale. She dozes easily, but can be aroused and made a little fretful by handling. She is breathing very fast. *Eyes.*—The pupils are equal and react to light. The left eye has a slight external strabismus. Ocular motions are coördinate. *Nose.*—No secretions, no excoriation. *Ears.*—Normal. *Mouth.*—The tongue is heavily coated, grayish yellow. The pharynx is reddened; the tonsils are much enlarged. *Neck.*—There is flaccid paralysis of the anterior and most of the posterior muscles. Attempt at flexion causes pain. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are enlarged. *Thorax.*—Well formed; respiration almost entirely thoracic. *Lungs.*—Clear. *Heart.*—Rapid, regular. *Abdomen.*—Retracted in inspiration; soft. Liver and spleen not enlarged. *Extremities.*—Upper: there is flaccid paralysis of the entire right arm, and of the left shoulder and arm. There is some power in the forearm (flexors). Wrist drop is present. Lower: the reflexes are absent on both sides. Kernig manipulation is painful, but resistance to it is voluntary. The left quadriceps is weak. *Temperature.*—101° F.

September 11. Spinal fluid: 20 c.c. clear limpid fluid; pressure not increased; cells, 174 per c.mm.; globulin, 0; sugar, +; polymorphonuclears, 3 per cent.; mononuclears, 97 per cent. Blood count: leukocytes, 26,300; differential count of 200 cells: polymorphonuclears, 70 per cent.; lymphocytes, 16.5 per cent.; large mononuclears, 3 per cent.; transitionals, 9 per cent.; basophiles, 0.5 per cent.; eosinophiles, 0.5 per cent.; stimulation form, 1 per cent.

September 12. This morning the temperature is 103° F. The child is breathing rapidly and almost entirely with the thorax. The alæ nasi are working more than they were last night. There is no advance of paralysis. The child is very ill.

September 13. The temperature at 8:00 A. M. is 100.8° F. Breathing is rapid and slightly irregular, purely costal in type. The accessory muscles of respiration are not brought into play. The ribs move well, the costal border flaring well out. Condition of paralysis unchanged.

September 14. The patient is not so well this morning. There is a faint tinge of cyanosis on the lips and a duskiness over the forehead. The alæ nasi are working strongly. Accessory respiratory muscles in the neck are beginning to work. The thorax still expands well, but the impression is given that the musculature is becoming fatigued. The patient has rather a fixed distant expression in the eyes, and occasionally the lids droop and the eyeballs roll slowly upward, sideways, and back again. The temperature continues between 101° and 102° F. The pulse rose to 160 this morning. It is small and very weak. Throughout both sides of the chest there are numerous scattered moist and sibilant rales. Transient vasomotor phenomena are seen in the irregular flushings of the face.

Spinal fluid: 15 c.c. of clear fluid; pressure not increased; cells, 44 per c.mm.; largely mononuclears; globulin, 0; sugar, +.

September 15. This morning the patient seems perhaps a little brighter than yesterday. The costal breathing is as strong as ever. The cyanosis is distinctly less. The alæ nasi are working as before. There is slightly more marked impairment of resonance over the left upper chest anteriorly and in the left axilla than on the right. The adventitious sounds consist chiefly of loud squeaks and coarse sounds. Posteriorly the rales predominate and are of a distinctly moist character. There are also many scattered rales over the right lateral and

posterior aspects of the chest. There is a slight expiratory grunt. The temperature continues between 101° and 102° F. The pulse rate is high and is stronger this morning.

8.30 P. M. Numerous rales can be heard, and over the lower axilla there is a distinct bronchial quality to the breath sounds. The percussion note is less dull on the left than it was this morning. Weakness of the muscles of deglutition is very marked.

September 16. The patient is much worse this morning. She is pale with a bluish tinge on the lips and general duskiness. The breathing is shallower and very rapid. The patient is more apathetic. The sounds in the chest are chiefly squeaks and coarse rales. It seems now like a generalized bronchitis of the larger tubes. She coughs occasionally, a loose cough.

About an hour later, the patient suddenly became much worse. The respiration was shallow, heart rapid and weak, and death followed. The heart continued to beat for some minutes after respiration ceased. Spinal fluid removed post mortem: about 6 c.c. of clear limpid fluid; cell count, 118.8 per c.mm.; globulin, slightly +; sugar, +.

CASE 30.

John A., age, 13 months. New York City. Admitted, October 29, 1911. Died, November 4, 1911. *Diagnosis.*—Acute poliomyelitis, bronchopneumonia.

Family History.—Negative. Habitat: the family lives in a rather dirty tenement on East 17th Street. They have three rooms. There are about twenty children in the house. No sickness of any kind is known in the house or neighborhood. There are no lame children in the neighborhood.

Past History.—The child has never had any illness.

Present Illness.—Five days ago, Tuesday, October 24, the baby seemed somewhat ill. He was quiet and would not play. The mother gave him castoria and on Wednesday he seemed perfectly well again. On Thursday he again seemed ill and was very quiet. He was worse on Friday and stayed in bed all day. That night, that is, thirty-six hours ago, the mother noticed that he did not move his legs. Yesterday he was no better and last night it was noticed that he preferred to lie with his head well back. This morning, Sunday, a doctor was called in and diagnosed the case as poliomyelitis. There has been no cough, diarrhea, constipation, or sweating, no nervous excitement. The child has not been very cross and has cried very little. There has been no stupor.

Physical Examination.—The patient is a pale, but healthy looking child, who lies quietly on his back. He is not very drowsy but becomes fretful and whines if handled. *Eyes.*—Pupils are equal and react normally. *Nose.*—No discharge. *Ears.*—Normal. *Neck.*—The neck is stiff. The patient can hold his head up in the sitting position, but prefers to let it fall back. *Superficial lymph nodes.*—The posterior cervical, axillary, and inguinal nodes are palpable. Many are enlarged. *Thorax.*—Well formed; respiratory movements normal. *Heart.*—Rapid but normal. *Abdomen.*—Not distended, no tenderness. Several small but distinct nodules can be felt in the central part of the belly. They do not feel like fecal masses and strongly suggest enlarged lymph nodes. Liver and spleen are not felt. *Extremities.*—Upper: there is distinct weakness of the right deltoid. Lower: there is complete flaccid paralysis of both lower extremities. Knee jerks

and Achilles reflexes are absent on both sides. There is no Babinski reflex. Kernig manipulation is not resisted but causes pain. *Surface.*—There are numerous small scaling spots on the forearms, and over the legs are many pink dull spots about 5 to 10 mm. in diameter. They look like healed impetigo. There is also a smaller, more punctate eruption over the forehead and a few papules on the face. *Temperature.*—101.6° F.

October 29. Lumbar puncture today yielded a clear fluid apparently under somewhat increased pressure. Cell count, 90 per c.mm.; lymphocytes, 92 per cent.; polymorphonuclears, 6 per cent.; large mononuclears, 2 per cent.; globulin, 0; sugar, + + (quick reduction).

October 30. The child is in good condition. He has considerable pain on moving the neck and legs. The left side of the mouth does not move quite so much as the right side does when the child cries. The left eye does not close so tightly as the right when the child cries. Lungs: there are generally scattered rales and a few piping sounds both anteriorly and more especially posteriorly. The child has a distinct grunting expiration at times. The temperature rose this morning to 102.2° F.

October 31. There are rales all through both sides of the lungs. In the right upper back the breath sounds are a little harsh. The upper right front is less resonant. Rales are everywhere coarse and bubbling.

November 1. Both sides of the chest are full of moist bubbling rales. The child looks badly. Respiration is almost wholly thoracic. There is inspiratory retraction of the abdominal wall, but when the thorax is compressed, the abdominal wall acts in a way that seems to indicate some weak diaphragmatic action

November 2. There are not so many moist bubbling rales this morning, but numerous squeaks. There is a good deal of mucus in the throat, and the child frequently gives a loose bubbling cough. There is very definite and almost complete weakness of the diaphragm. The child looks pale and possibly very faintly dusky. The weakness of the right deltoid has advanced to complete paralysis of practically the whole extremity. Only weak action of the muscles of flexion of the fingers remains. Possibly the left deltoid is also weak. The temperature was 101° F. at noon.

November 3. The child has a high temperature this morning. Both sides of the chest are full of rales, coarse and bubbling. The diaphragm is hardly working at all. The child looks very badly. The temperature is 104.4° F.

P. M. The child has been out of doors all day. The physical condition is unchanged except that there are, if anything, fewer rales in the chest. The pulse is good.

November 4. Last evening the child looked rather badly, but the pulse was of good quality and not very rapid. When seen at 2:00 A. M. the condition was unchanged. At 6:00 A. M. just after the nurse had felt the pulse and found it unchanged, the child suddenly died.

CASE 31.

Max G., age, 4¾ years. New York City. Admitted, October 18, 1911. Discharged, November 11, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Negative. Habitat: the family lives in an eighteen family tenement. The house is new and is clean and airy. There are cockroaches, but

no other parasites in the house. The family have no cats or dogs. The father knows of no possibility of contact.

Past History.—The child has always been well and strong.

Present Illness.—On Sunday morning, October 15, three days ago, the child was perfectly well. That evening he had a headache and a little fever. The next morning he was feverish and vomited. Since that time he has been in bed. Monday evening the child had pain on moving his neck. He has not sweat much and has had no convulsions. Bowels and urination have been normal. On the first day of his illness the child was very drowsy, but he has been getting brighter. On Monday, two days ago, it was noticed that he was unable to move his eyes so as to look downward. He could close the eyes and move them normally in other directions. No other muscular weakness has developed. The child swallows normally but eats little. He has had fever each night.

Physical Examination.—The patient is a well built, rather thin boy, lying on his left side with his legs curled up. Both eyes are partly closed, leaving a slit about 0.5 cm. in width in which the sclera and lower edge of the cornea of the upturned eyes are visible. The child mutters from time to time, but it is difficult to understand him. He responds weakly to his name and answers simple questions, but he is very drowsy and when left alone lies in a semistuporous condition. The skin is hot and dry. The face is symmetrical, is not flushed, and there is no rash. *Eyes.*—The eyelids close normally when the patient is told to close the eyes. When let alone his eyes are held closed. There is marked photophobia. The pupils are equal and react quickly to light and accommodation. Both eyes move upward, and to the right and left normally, but neither eyeball can be made to look downward. He cannot see anything below the level of the eye. There is no strabismus. He can count his fingers quickly and correctly. *Ears.*—No discharge, no tenderness over the mastoids. *Nose.*—No discharge or excoriation. *Mouth.*—The lips and teeth are dry. The mouth is held partly open. The tongue is slightly coated, protrudes straight and for a good distance. Both tonsils are enlarged and ragged, but not reddened. The pharynx is not red. *Superficial lymph nodes.*—There is moderate enlargement of the glands in the neck, axillæ, and groins. *Neck.*—The neck is not retracted but it is held stiffly, and resists all attempts at flexion. *Chest.*—Well formed, symmetrical, moves normally with respiration. There is no rosary. *Lungs.*—Clear throughout on auscultation and percussion. *Heart.*—Not enlarged, regular, sounds of good quality. The pulmonic second is louder than the aortic second. A soft systolic murmur is heard over the precordium. *Abdomen.*—Full, soft, tympanitic, no masses or tenderness. Abdominal reflexes are active. There is no tache cérébrale. The abdominal wall moves normally with respiration. Abdominal muscles are strong. Cremasteric reflexes are active. *Extremities.*—Movements are normal and strong on both sides. Knee jerks on both sides are active when obtained, but are hard to obtain. Achilles reflexes are absent on both sides. Kernig's sign: when the legs are extended a little more than a right angle, he complains of pain and tightens up his hamstrings. The Babinski test is unsatisfactory, first an extension and then a flexion. Oppenheim test: a slight but suggestive extension of the big toe on both sides was obtained. There is no tenderness on pressure over the legs. Both arms move strongly and normally. Arm reflexes are not obtained. *Temperature.*—104.2° F.

Spinal fluid: clear, colorless fluid; cell count, 61.1 per c.mm.; differential count of 200 cells: polymorphonuclears, 22.9 per cent.; mononuclears, 78 per cent.; globulin, 0; sugar, slightly +.

October 19. The patient lies with a peculiar expression in his eyes like a fixed stare, but he says he feels much better than yesterday. He follows an object with his eyes until the object is moved downwards. Then he says, "I can't look down." There is slight external strabismus of the left eye. The pupils react normally. There is no paralysis of upper or lower extremities. The knee jerks are hard to obtain, but when they appear they are distinctly increased. Achilles jerks are both present. The Babinski reflex on the right is distinctly suggestive. The Oppenheim reflex is positive. There is marked ataxia of arms and legs. The patient contracts the hamstrings, and resists extension of the leg when Kernig manipulation is attempted.. It is apparently quite painful.

Blood count: leukocytes, 13,000; differential count of 200 cells: polymorphonuclears, 51.5 per cent.; lymphocytes, 37 per cent.; large mononuclears, 7 per cent.; transitionals, 4.5 per cent.

October 20. Spinal fluid: clear watery; cells, 320 per c.mm.; sugar, +; globulin, very slightly +. Smear: a few red cells; polymorphonuclears, 66 per cent.; mononuclears, 34 per cent.; no organisms seen.

Note by Dr. Schirmer: "Position of the eyes when the boy looks in the distance is slightly divergent. Motion to the right and left side, and upward, is possible to physiological limits. In looking downward there is a marked restriction which is equal in both eyes. They can be moved downward to about 25° below horizontal. When the boy looks at a distance of one meter at my finger and I move the finger toward him, he is not able to converge at all. One of the eyes begins to deviate, and the movement develops into an associated one to one side."

October 21. His condition is about the same. The child can look down a little better. He is irritable and wants to be let alone. When he is asleep he has frequent jerky movements of the arms. Reflexes of the legs are the same as yesterday except that the knee jerks and Achilles reflexes are both more active.

October 23. The child is much better. There is slight strabismus (external on left?). The child can look down slightly better than before, especially with the right eye. Knee jerks are very active, Achilles reflexes active, Babinski negative, Oppenheim suggestive on both sides. Kernig's and neck signs are less painful.

Spinal fluid: 8 c.c. of clear limpid fluid; pressure not increased; cells, 6 per c.mm.; globulin, normal; sugar, +.

October 25. Eye grounds are negative.

October 26. Spinal fluid: 10 c.c. of clear, watery, colorless fluid; pressure not increased; cells, 25 per c.mm.; mononuclears, +; globulin, very slightly +; sugar, +.

October 31. Spinal fluid: 20 c.c. of clear, non-opalescent, watery fluid; cells, 11 per c.mm.; mononuclears, +; globulin, very slightly +.

November 4. The patient walked today. He showed a peculiar unsteadiness which at first looked like ataxia. On close examination, however, there is seen to be a distinct element of spasticity in the gait. The knee jerks are both exaggerated. The Achilles jerks are hard to obtain and certainly not exaggerated. The eye condition is unchanged. The patient cannot converge.

November 11. The general condition is excellent. The patient's gait is now quite steady. Eyes: the tendency is for him to roll his eyes up, but he can move them both down almost normally. There is a slight external squint of the left eye. He does not converge. He seems to focus with his right eye, and if this is closed, he has to rotate the left eye inward in order to see an object. Both knee jerks are exaggerated, both Achilles jerks are nearly absent, abdominal and cremasteric reflexes are very active. Kernig manipulation is still painful. Oppenheim and Babinski tests are negative.

CASE 32.

Patrick T., age, 23 months. New York City. Admitted, October 4, 1911. Discharged, October 15, 1911. Result, improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—One brother, aged five years, had tonsillitis seven days ago and now has a cold in the head. He has had no muscular weakness or drowsiness. One sister, aged four years (Hannah T.), was admitted yesterday to Ward 1, with poliomyelitis. Another sister, aged three years, had a cold one week ago, lost her appetite and was drowsy for two days, but is now perfectly well. She has had no muscular weakness. A third sister, eight weeks old, is healthy. Habitat: the family lives on Lexington Avenue in a flat; they have five rooms, second floor front. The light and air are good. The mother and father, the sister with poliomyelitis, and the patient slept in the same room, the patient sleeping in a crib by himself and the sister in bed with the father and mother. The other three children sleep in a room together. There are flies, mosquitoes, bedbugs, and roaches in the house. The mother knows of no similar illness in the neighborhood.

Past History.—The patient has always been a strong healthy child.

Present Illness.—October 3, two days after the onset of the sister's illness, the mother noticed that the child was drowsy. This morning she noticed that he was "shaky" in his legs and could not rise from a sitting position. When placed on his feet he could walk fairly well, but dragged one leg. He has been able to use his arms well, and can feed himself. The mother has noticed no fever, but drowsiness has continued, and the child has been irritable. There has been no vomiting, no convulsions, and no retraction of the neck. Bowels and urination have been normal. The child had a cold and discharge from the nose one week ago, but it lasted only two days.

Physical Examination.—The patient is a well nourished, sturdy child. He is drowsy but can be easily awakened and then becomes irritable. On being let alone he quickly goes to sleep again. *Head.*—Well formed, forehead slightly prominent. *Face.*—Symmetrical in repose, not drawn to either side on crying. Both eyes can be closed. *Eyes.*—Conjunctivæ and scleræ are clear. Eye slits seem equal. There is a tendency to keep the eyes closed and when open to hold them away from the light. External ocular movements are good, no strabismus. Pupils are equal, moderately dilated, and react rather slowly to light. *Ears.*—No mastoid tenderness, no discharge. External ear, and meatus on the left side are reddened. Drums on both sides are obscured by wax. *Mouth.*—Lips and mucous membranes are of fair color. The tongue has a heavy yellowish white coat. Pharynx and fauces are red and injected. The tonsils are large and irregular; no exudate. *Neck.*—There is no retraction of the neck. The head is

easily held erect. There is no muscular weakness. The child resists anterior flexion slightly. *Superficial lymph nodes.*—The cervical, axillary, and inguinal glands are all easily palpable. *Chest.*—Well formed, expansion good, both sides move equally in respiration, diaphragm active. *Lungs.*—Clear on ausculation and percussion. *Heart.*—Action not especially rapid, sounds well heard, regular, no murmurs. The heart is not enlarged. *Abdomen.*—Soft, moves well with respiration, no masses or tenderness, no bulging. Abdominal muscles are strong. *Extremities.*—Upper: arm movements are normal, muscles show no weakness. Lower: the legs can be moved in all directions. The child stands alone and can walk well. The legs can be fully flexed on the abdomen without causing pain. The child, however, shows some irritability on being handled in this way. *Reflexes.*—Knee jerks, Achilles, and abdominal reflexes are active. Babinski and Kernig reflexes are absent. *Temperature.*—100.6° F.

October 4. Spinal fluid: 35 c.c. of clear limpid fluid; pressure definitely increased (child crying somewhat); cells, 94 per c.mm.; mononuclears, 93 per cent.; polymorphonuclears, 7 per cent.; globulin, 0; sugar, +.

October 5. Blood count: leukocytes, 15,000; differential count of 200 cells: polymorphonuclears, 52.5 per cent.; lymphocytes, 31.5 per cent.; large mononuclears, 13.5 per cent.; transitionals, 1.5 per cent.; basophiles, 0.5 per cent.; eosinophiles, 0.5 per cent.; stimulation form, 0.

October 8. There is no evidence of paralysis. Reflexes are present and active in the legs. The child can walk and stand well, but his gait is rather wobbly. He is irritable and cries a good deal, but it seems to be temper chiefly. His legs appeared weak, and he fell when first put on the floor, but when he was started walking towards his sister, he could walk very well.

October 15. The child walked very well this morning, several times up and down the hall. Both knee jerks and Achilles reflexes are active. The lungs are clear. The tonsils are both a little enlarged, but not especially red. Moving the legs (Kernig) seems to be painful still, though it is hard to distinguish the pain from temper. The child is discharged today.

CASE 33.

Helen B., age, 6 years. Bergenfield, N. J. Admitted, September 2, 1911. Discharged, September 17, 1911. Result, much improved. *Diagnosis.*—Acute poliomyelitis (abortive form).

Family History.—Negative.

Past History.—The patient has always been a healthy child and was never sick until July when she had whooping cough. She has always played with Helen K. (see case 29, page 174, who was taken sick on August 16 with acute poliomyelitis and died on August 21).

Present Illness.—The child was perfectly well yesterday morning, September 1. She came down to breakfast singing, ate her breakfast well, and played all the morning. She also ate a hearty lunch and played for a while in the afternoon. Later in the afternoon she complained of headache, which continued all the evening. She ate no supper, slept fairly well, was not very restless but seemed dull. This morning she ate no breakfast. She has not vomited, but there was no movement of the bowels yesterday. No weakness of any extremity has been noticed. She has had some pain in the back of the neck and forehead.

Physical Examination.—The patient is a well developed little girl who lies quietly in bed and looks acutely sick. The face is flushed, the expression of the eyes is heavy and the lids are half closed or closed most of the time; she is very apathetic. *Eyes.*—The pupils are equal and react normally. Scleræ are clear, ocular motions normal. *Nose.*—No discharge. *Face.*—There is no weakness of the face muscles. *Ears.*—Normal. *Mouth.*—Buccal mucous membranes are clear. The teeth are in fair condition. No Koplik spots. The tongue has a very light greyish coat. Pharynx and tonsils are slightly reddened. The latter are large, especially the right. *Superficial lymph nodes.*—The posterior cervical, submental, axillary, and inguinal nodes are palpable. *Neck.*—Not stiff. *Thorax.*—Well formed, respiratory motions are normal. *Lungs.*—Clear. *Heart.*—Rapid, sounds normal, action regular. *Abdomen.*—Not distended, soft, no tenderness, no masses. *Extremities.*—Upper: normal, no tenderness. Lower: there is good power in all the muscles, no tenderness. Kernig manipulation causes no pain. Knee jerks are very active on both sides; crossed adductor reflex is obtained on tapping the left patellar tendon. The Achilles reflex is also active, the Babinski reflex negative on both sides. *Back.*—No weakness, no pain or tenderness. *Surface.*—There are several fresh bruise marks over the legs. *Temperature.*—103.8° F.

September 2. Blood count: leukocytes, 8,800; differential count of 200 cells: polymorphonuclears, 77 per cent.; lymphocytes, 17.5 per cent.; large mononuclears, 0.5 per cent.; transitionals, 4 per cent.; stimulation form, 1 per cent.

Spinal fluid: 20 c.c. of almost clear limpid fluid. There is a faint suggestion of opalescence; cells, 5 per c.mm.; polymorphonuclears, 20 per cent.; mononuclears, 80 per cent.; globulin, 0; sugar, +.

September 3. The patient slept well. Her temperature is 99.1° F. This morning the temperature has fallen to 99° F. She is still rather listless but much less dull than yesterday. She has no headache. The knee jerks are still very active. The patient says she feels much better. She vomited after taking citrate of magnesia. In the afternoon the mother came and finding the child's temperature normal insisted on taking her home. She was advised to leave the patient but would not. The temperature was 98.7° F. The patient showed some unsteadiness on her feet but no definite weakness. About an hour later the mother returned in a taxicab with the child. On the way to the train she had complained bitterly of her head and vomited. The child begged to be put in bed. She looks greatly prostrated. There is a distinct tendency to keep the head back, and she assumes the position on her side so as to facilitate this. The neck is very slightly stiff, and an attempt to flex it causes pain. The Kernig manipulation causes pain about the knees. The knee jerks are still exaggerated.

Spinal fluid: about 2 c.c.; cell count, 62 per c.mm.; polymorphonuclears, 11.3 per cent.; mononuclears, 88.7 per cent.; globulin, slightly +.

Blood count: leukocytes, 11,200; differential count of 200 cells: polymorphonuclears, 79.5 per cent.; lymphocytes, 14.5 per cent.; large mononuclears, 0.5 per cent.; transitionals, 4.5 per cent.; basophiles, 0; eosinophiles, 0.5 per cent.; stimulation form, 0.5 per cent.

September 4. Temperature: 99.1° F. The patient seems a little better this morning. She is still apathetic and prefers to lie on her side. The neck resists flexion slightly, and the attempt to flex it causes pain in the occipital region.

Kernig manipulation causes slight pain in the knee. Knee jerks are not quite so exaggerated as before, but are still very active. No weakness is found in any muscles. The patient sits up well. The tongue has a heavy yellowish grey coating.

P. M. She has been drowsy and sleepy most of the day. When her father came the child paid hardly any attention to him and showed no signs of pleasure. She answered his questions only by nods and shakes of the head. As soon as he left the room she became drowsy again. At 3:00 P. M. the knee jerks are found to be so much less active than before that several blows of the hammer are required to elicit the jerk. They are fairly active when they do respond. No weakness is found. The neck is a little less stiff.

10:20 P. M. The patient is sleeping, but half waked during the process of eliciting knee jerks. They are less easily obtained than when last noted. When they respond it is with about normal activity. The stiffness of the neck late this afternoon (about 5:20 P. M.) had almost entirely gone.

September 5. The patient slept well this morning. She seems brighter, though still not very communicative. She has no headache. The neck is not stiff. Both knee jerks are active. She still prefers to lie in bed and does not feel energetic. The von Pirquet reaction made two days ago is positive, a raised urticarial-like area about 1 cm. in diameter, and red. The temperature dropped to normal last night. This morning it is 99° F. again.

P. M. The patient continues to be somnolent and takes very little interest in her surroundings. There is no change in her physical condition. It is possible that the right palpebral fissure is somewhat narrower than the left. Examination of the eye grounds reveals no abnormality.

September 6. The patient is a little brighter this morning but still prefers to lie in bed. The tongue has almost entirely cleared. The neck is a little stiff. "Signe de la nuque" is present. The left knee jerk is obtained on reinforcement only, right knee jerk normal.

Spinal fluid: about 8 c.c. of not quite clear, slightly opalescent fluid with faintly yellowish tinge; cells, 59 per c.mm., mostly mononuclears; globulin, slightly +; sugar, +. In the wet preparation for counting, a large phagocytic cell was seen containing five small mononuclears. Blood count: leukocytes, 5,600; differential count of 200 cells: polymorphonuclears, 68.5 per cent.; lymphocytes, 22 per cent.; large mononuclears, 1.5 per cent.; transitionals, 6 per cent.; basophiles, 0; eosinophiles, 1 per cent.; stimulation form, 1 per cent.

This evening the patient is somewhat brighter but continues to answer questions by shakes or nods and occasionally a monosyllabic reply. She is still drowsy. There is no change in her physical condition.

September 7. The patient seems better this morning. She sits in a chair easily, and also walks well, though perhaps with a little uncertainty. She continues to be most unresponsive. Her appetite is better, however, and she ate her supper last night, and breakfast this morning with a relish. There is still slight rigidity of the neck and "signe de la nuque." The knee jerks are the same as yesterday.

September 11. The patient is considerably better. She talks more and is losing her apathy. She still does not feel much like sitting up. The knee jerks are active. Spinal fluid: 20 c.c. of clear limpid fluid; pressure not increased; cell count, 4 per c.mm.; globulin, very slight haziness (normal); sugar, +.

September 13. Blood count: leukocytes, 11,800; differential count of 200 cells: polymorphonuclears, 78.5 per cent.; lymphocytes, 14.5 per cent.; large mononuclears, 4.5 per cent.; transitionals, 3.5 per cent.; eosinophiles, 0.5 per cent.; basophiles, 0; stimulation form, 0.5 per cent.

September 15. While the patient is better in every way, she still gives the impression of being a little apathetic. Her appetite is poor. Besides the unsteadiness that one would expect on first getting up, her manner of walking gave distinctly the impression of spasticity. This was quite in accord with the generally increased tone of the legs throughout her illness and with the exaggerated knee jerks. This morning the knee jerks are very much exaggerated.

September 17. The patient has been up in a chair for the past two days and was walking yesterday. She walks much more steadily. On physical examination nothing abnormal can be found but the still somewhat exaggerated knee jerks. The neck has no trace of stiffness and the "signe de la nuque" is gone. She feels well and is discharged.

CASE 34.

Clara T., age, 8 months. New York City. Admitted, August 22, 1911. Discharged, August 24, 1911. Result, not improved. *Diagnosis.*—Acute poliomyelitis.

Family History.—Mother and father are living and well. There is one other child in the family, a twin sister of the patient, who now has poliomyelitis in this hospital.

Past History.—The child has had no illness previous to the onset of the present trouble.

Present Illness.—The child was perfectly well until yesterday evening, August 21. At this time the mother noticed that she was rather irritable and objected to being handled. She vomited several times, and had a slight cough. Today she has again vomited several times and at four o'clock this afternoon her temperature was 102° F. When the child was examined in the receiving ward, handling of the right leg caused loud screaming.

Physical Examination.—The patient is a well nourished baby with good color, bright alert expression, and does not look sick. She lies quietly, and is sometimes playful. During examination she regurgitated suddenly and without effort. Occasionally she coughs. *Eyes.*—Pupils are equal and react normally. Ocular motions are normal, no squints. *Ears.*—Normal. *Nose.*—Normal. *Mouth.*—Buccal mucous membrane is clear. Tonsils are a little enlarged, pharynx slightly reddened. *Neck.*—Distinct rigidity. *Superficial lymph nodes.*—The posterior cervical and left axillary nodes are palpable. *Thorax.*—Well formed, respiratory motions normal. *Heart.*—Normal. *Lungs.*—Normal. *Abdomen.*—Well rounded and prominent, soft, moves properly with respiration. The spleen is easily felt. *Extremities.*—Upper: there is possibly a shade less strength in the right shoulder than in the left, but this is very indefinite. At times there is a distinct stiff shaking of the arms. Lower: knee jerks absent on the right, very faint reaction on the left; Achilles reflex absent on the right, present on the left; Babinski test negative on both sides; Kernig's sign: on the right side no resistance but some distress, on the left slight resistance. The left leg is moved about in a very active manner. The right, however, is not moved nearly so much, practically not at all spontaneously. When prodded, there is some move-

ment to withdraw the extremity, but this is chiefly in the lower leg muscles, and very little or not at all in the thigh. *Surface.*—There is a faint erythema over the upper part of the chest anteriorly. Over the inner end of the right eyebrow is a small superficial moist ulceration, sharply circumscribed and looking much like a collapsed water blister. *Temperature.*—102.6° F.

August 22. Spinal fluid: 30 c.c. of slightly greyish opalescent fluid; pressure at first much increased (child crying); later, slightly increased; cells, 990 per c.mm.; 95 per cent. polymorphonuclears. Smear: polymorphonuclears, 85.5 per cent.; transitionals, 4 per cent.; mononuclears, 10.5 per cent. (including probably both endothelial and lymphocytes, these cells being destroyed beyond recognition); globulin, +; sugar, + +. Blood count: leukocytes, 35,600; differential count of 200 cells: polymorphonuclears, 32 per cent.; lymphocytes, 55.5 per cent.; large mononuclears, 1 per cent.; transitionals, 9 per cent.; basophiles, 0; eosinophiles, 1 per cent.; stimulation form, 1.5 per cent.

August 23. Spinal fluid: about 7 c.c.. The first cubic centimeter was clear and slightly opalescent. This was used for cell count and smear. The smear contained a few red blood corpuscles, with great excess of mononuclears. Cell count, 627 per c.mm.; polymorphonuclears, 20 per cent.; mononuclears, 80 per cent.; no test for globulin, nor for sugar. On admission the child was irritable and cried a great deal. The knee jerks and Achilles jerks on each side were exaggerated. Both lower extremities were strong and active. This evening the left knee jerk is gone. The left Achilles jerk is very faintly present. The child cannot move the left thigh now, so that both lower extremities are affected. The flexors and extensors of the feet are still acting, but are weak. The child is rather more fretful this evening.

August 24. This morning there is no further advance of the paralysis, but both lower extremities are flaccid, save for some slight motion in the flexors and extensors of the feet and toes. The Achilles jerk on the left is gone. The arms are active and strong. The temperature has risen again to 102.8° F. The child looks bright, but is perhaps too alert and nervous. She is still inclined to be irritable if handled. If a sudden noise occurs, she blinks rapidly for several seconds and starts. Blood count: leukocytes, 23,200; differential count of 200 cells: polymorphonuclears, 49 per cent.; lymphocytes, 33.5 per cent.; large mononuclears, 5 per cent.; transitionals, 9.5 per cent.; basophiles, 0.5 per cent.; eosinophiles, 0.5 per cent.; stimulation form, 2 per cent.

This afternoon the child seems to be in about the same condition. It is possible that the patient is slightly more somnolent and does not show the nervous activity of the upper extremities that she did yesterday. Spinal fluid: a few cubic centimeters of slightly turbid, opalescent fluid were obtained; cell count, 1,221 per c.mm. In the wet count there are many very large phagocytic mononuclear cells with vacuoles; mononuclears, 92 per cent.; globulin, + (moderate flocculent precipitate); no test made for sugar. The father insisted on taking the child home this afternoon.

EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Interstitial meningitis in the anterior fissure of the cord, with beginning perivascular infiltration of the gray matter (human).

Fig. 2. Infiltration with small round cells in the perivascular lymph spaces (monkey).

Plate 2.

Fig. 3. Diffuse areas of hemorrhage in the gray matter of the cord (human).

Plate 3.

Fig. 4. Neurophages in nerve cells of the anterior horn (monkey).

Fig. 5. Extensive infiltration of the gray matter of the cord (human).

Plate 4.

Fig. 6. The inflammatory process is seen extending throughout the whole anterior horn (monkey). Low power.

Fig. 7. Drawing of the lumbar cord of a monkey showing extensive interstitial meningitis and beginning perivascular infiltration.

Plate 5.

Fig. 8. Perivascular and diffuse round cell infiltration in the medulla (monkey).

Plate 6.

Fig. 9. Cervical ganglion, showing advanced inflammatory reaction.

Plate 7.

Fig. 10. A spinal ganglion showing diffuse inflammatory process and beginning necrosis of the nerve cells (human).

Plate 8.

Fig. 11. Neurophages in a spinal ganglion (monkey).

Fig. 12. Human lymph node. The pale centres of the nodules are formed by large endothelial cells. Low power.

Plate 9.

Fig. 13. Human liver. Drawing showing necrosis of the liver cells and accumulation of lymphoid and polynuclear cells. High power.

Fig. 14. Electrocardiogram of the arrhythmia regularly seen in the fatal cases.

Plate 10.

Fig. 15. Photograph of a case with profound stupor, showing irritated expression. The paralyzed left arm has been flung over by the shrugging of the shoulder. The retraction of the neck is also apparent.

FIG. 16. A case of intercostal paralysis, showing protrusion of the abdomen by contraction of the diaphragm and concomitant retraction of the thorax.

FIG. 17. Front view of patient shown in figure 2 with intercostal paralysis.

PLATE 11.

FIG. 18. Paralysis of the lateral abdominal muscles. Bulging is produced by crying.

FIG. 19. Paralysis of the lateral abdominal muscles and right rectus. Bulging is caused by the attempt to sit up.

PLATE 12.

FIG. 20. The only evidence of paralysis is in the upper distribution of the seventh nerve. This was a profoundly stuporous case.

PLATE 13.

FIG. 21. Residual facial palsy in a case with profound stupor.

FIG. 22. The same as figure 21.

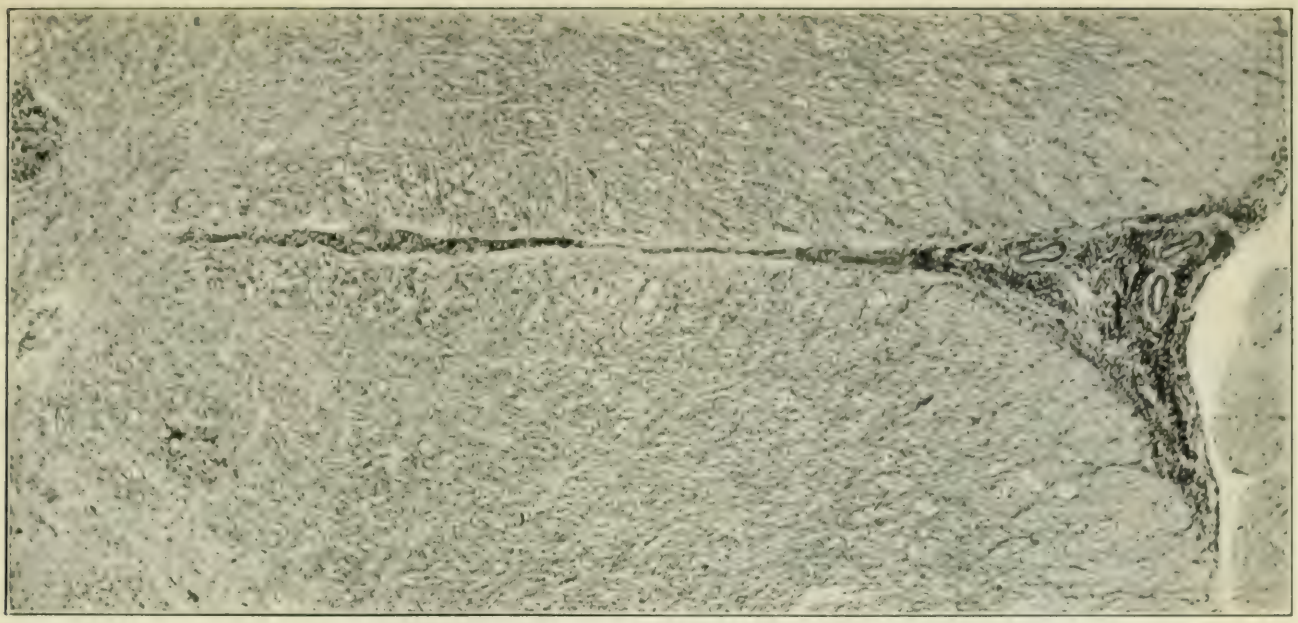


FIG. 1.

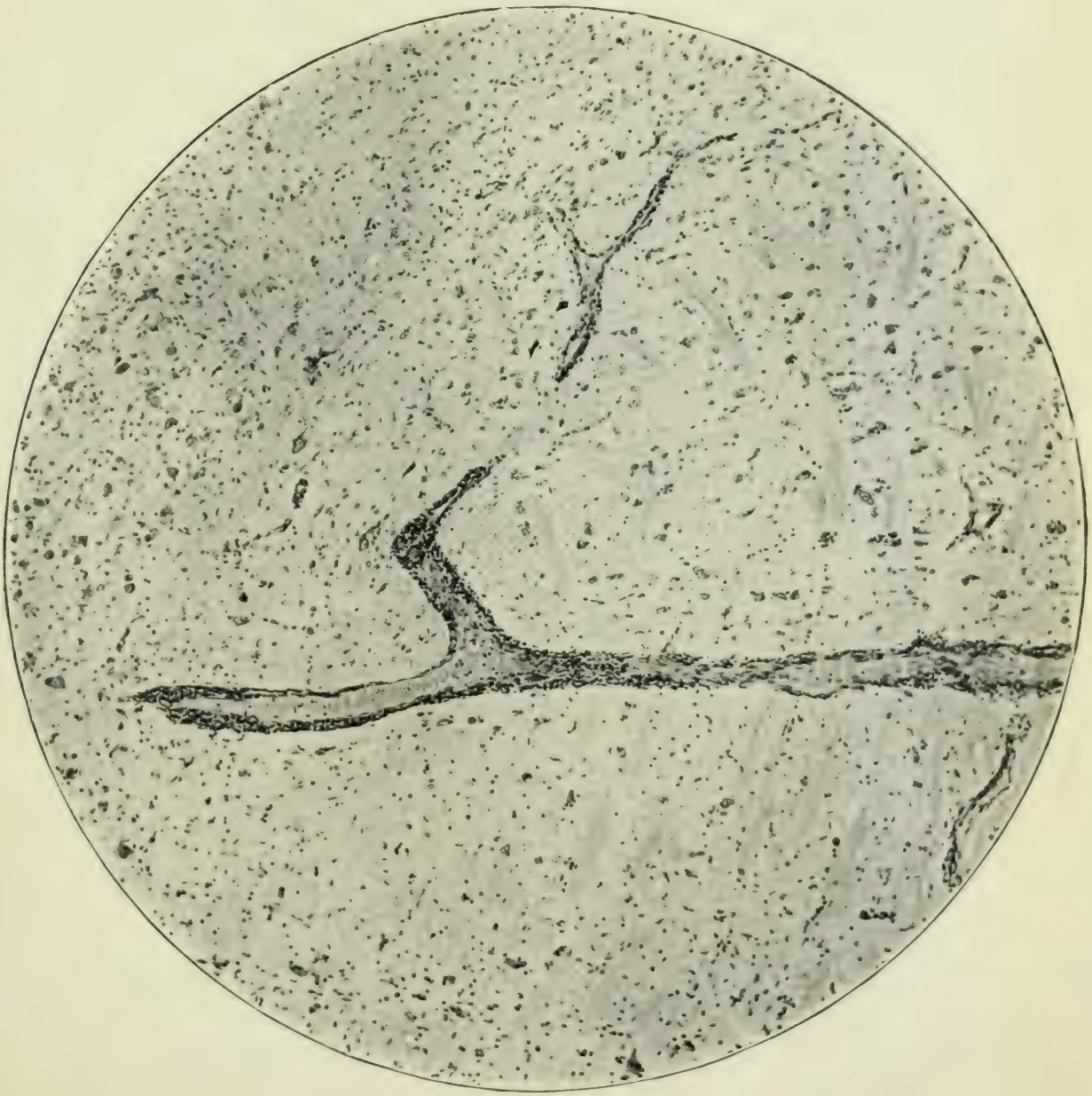


FIG. 2.

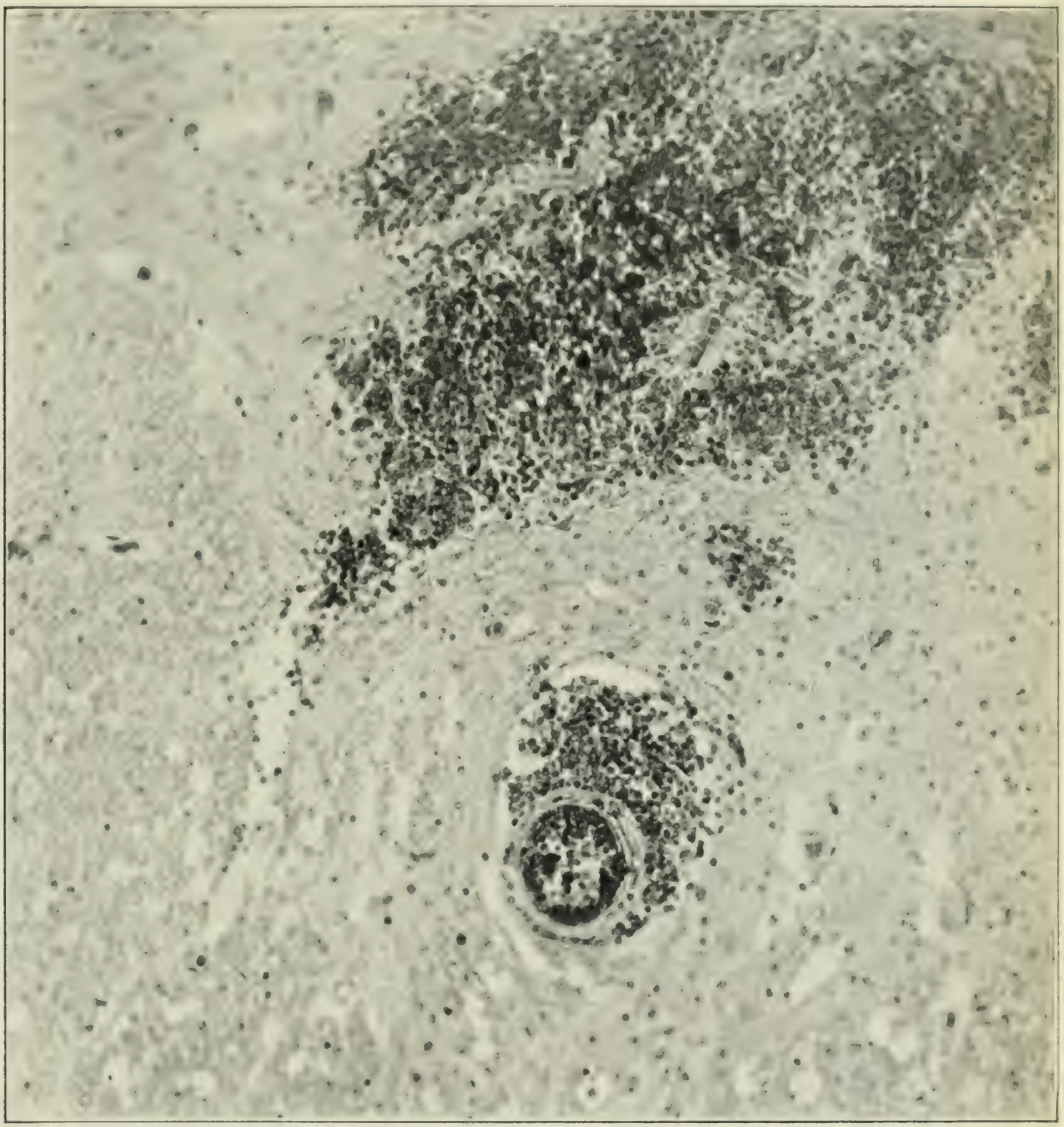


FIG. 3.

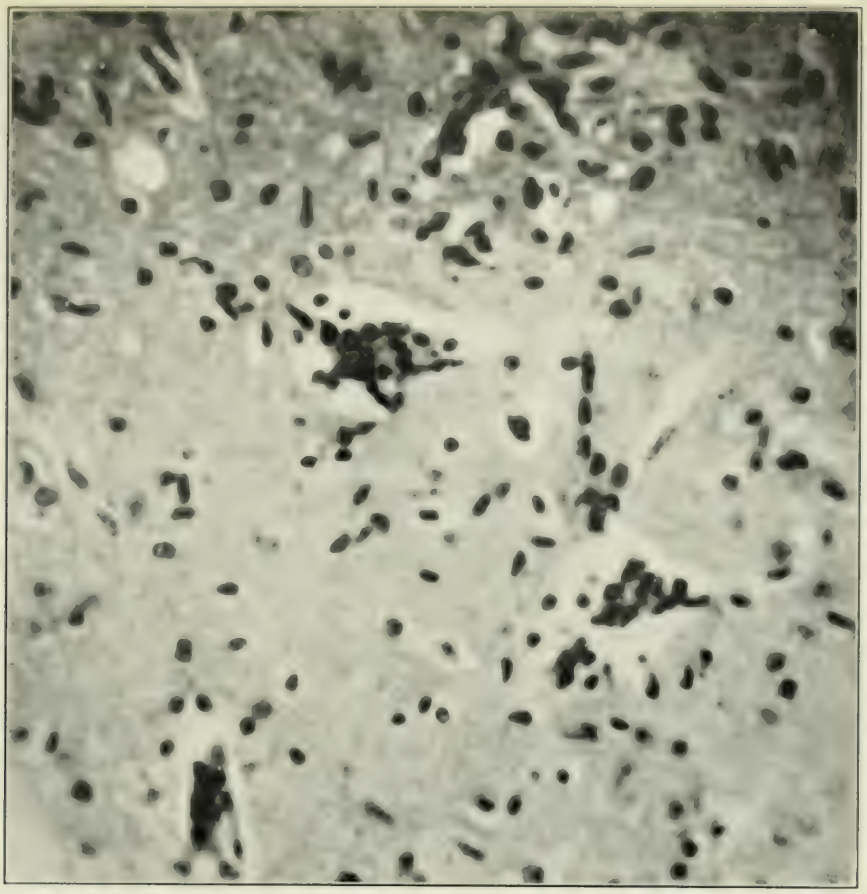


FIG. 4.

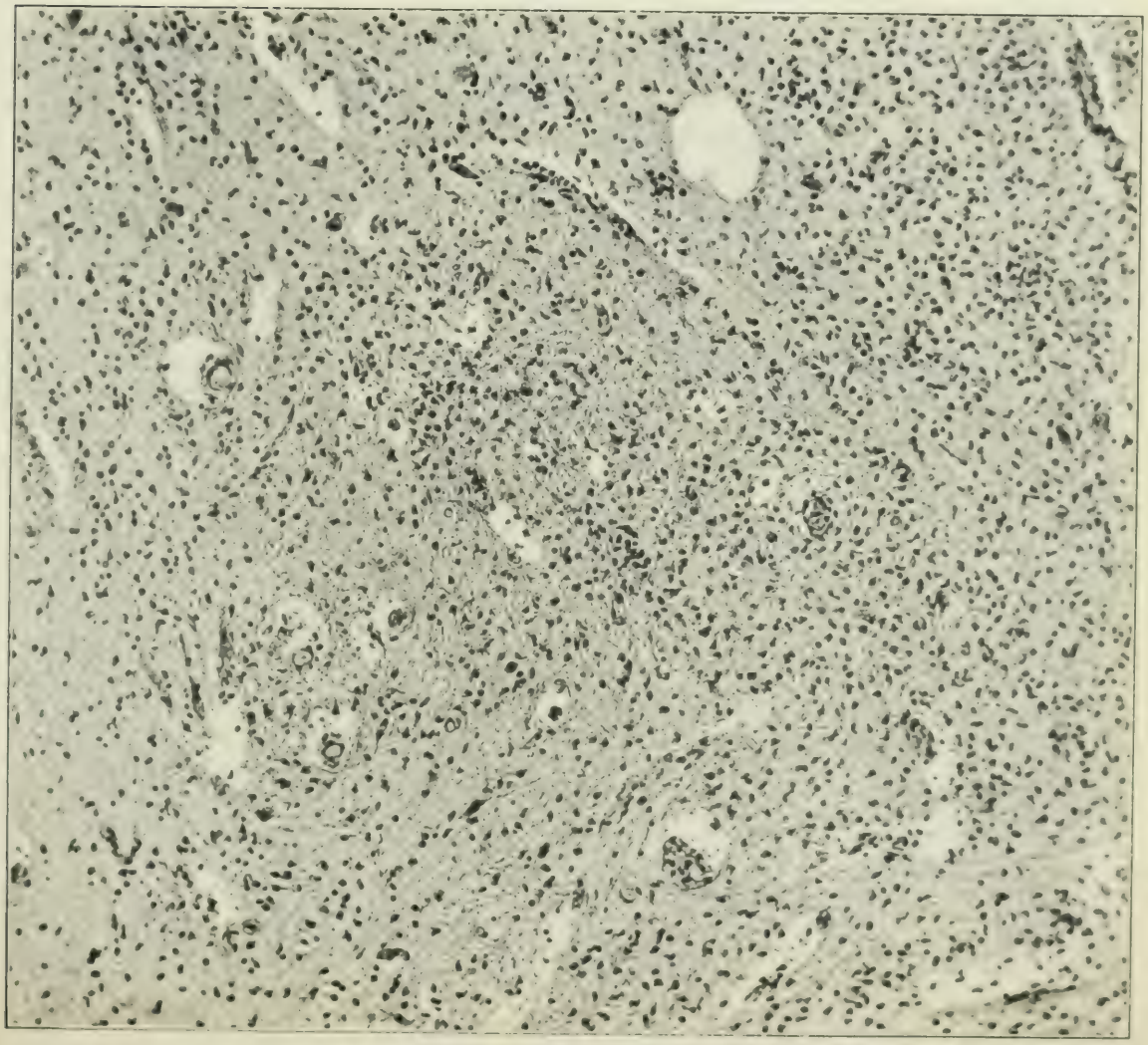


FIG. 6.

FIG. 8.

FIG. 9.

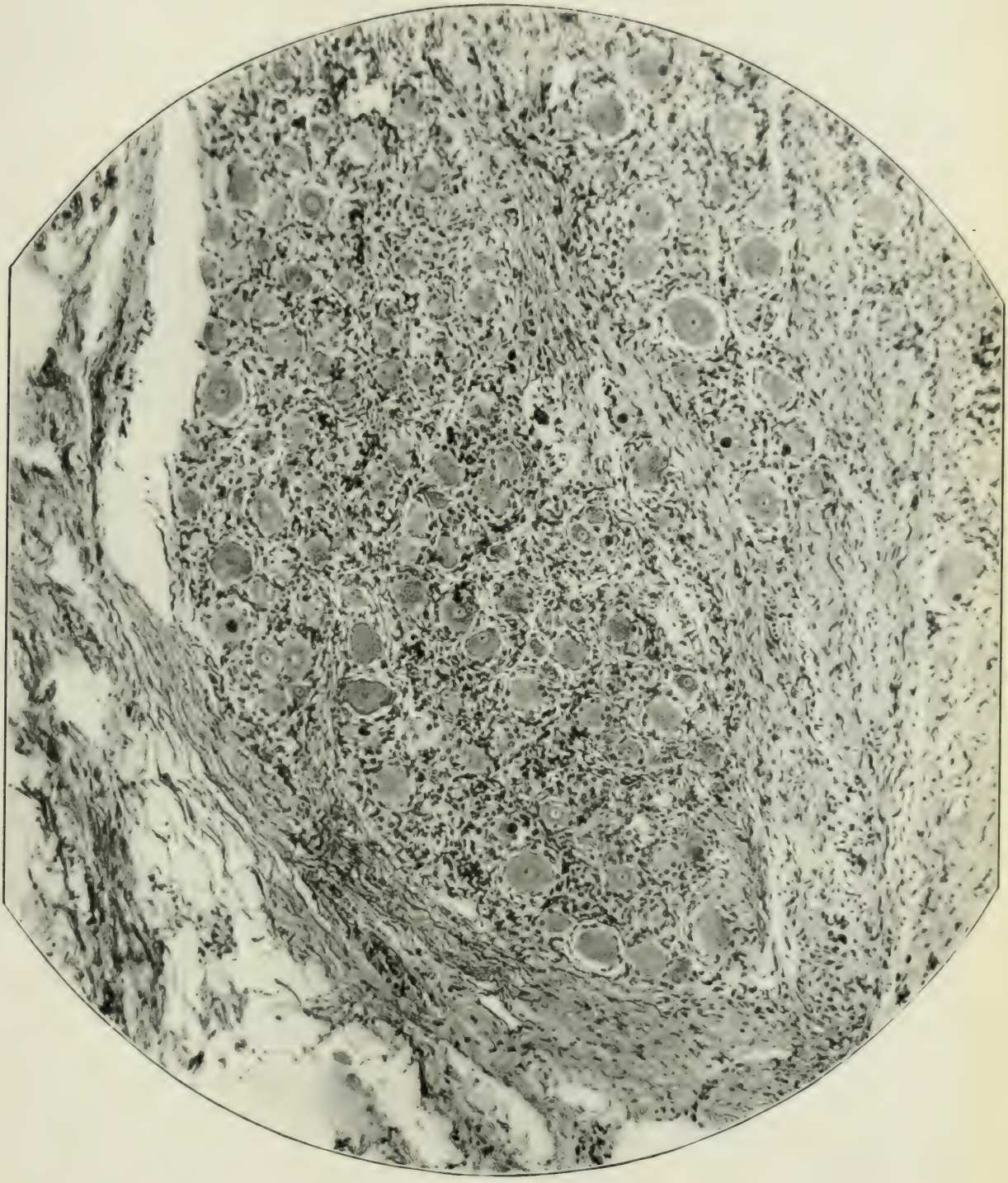


FIG. 10.

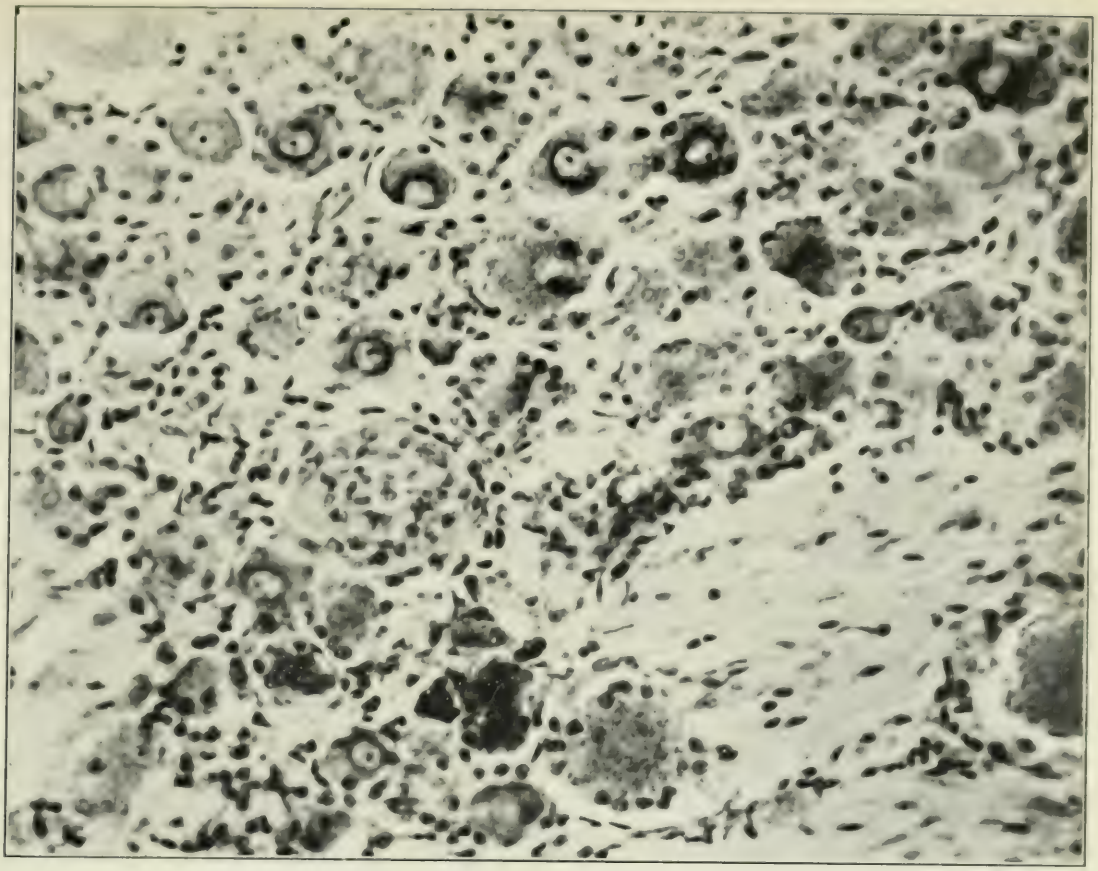
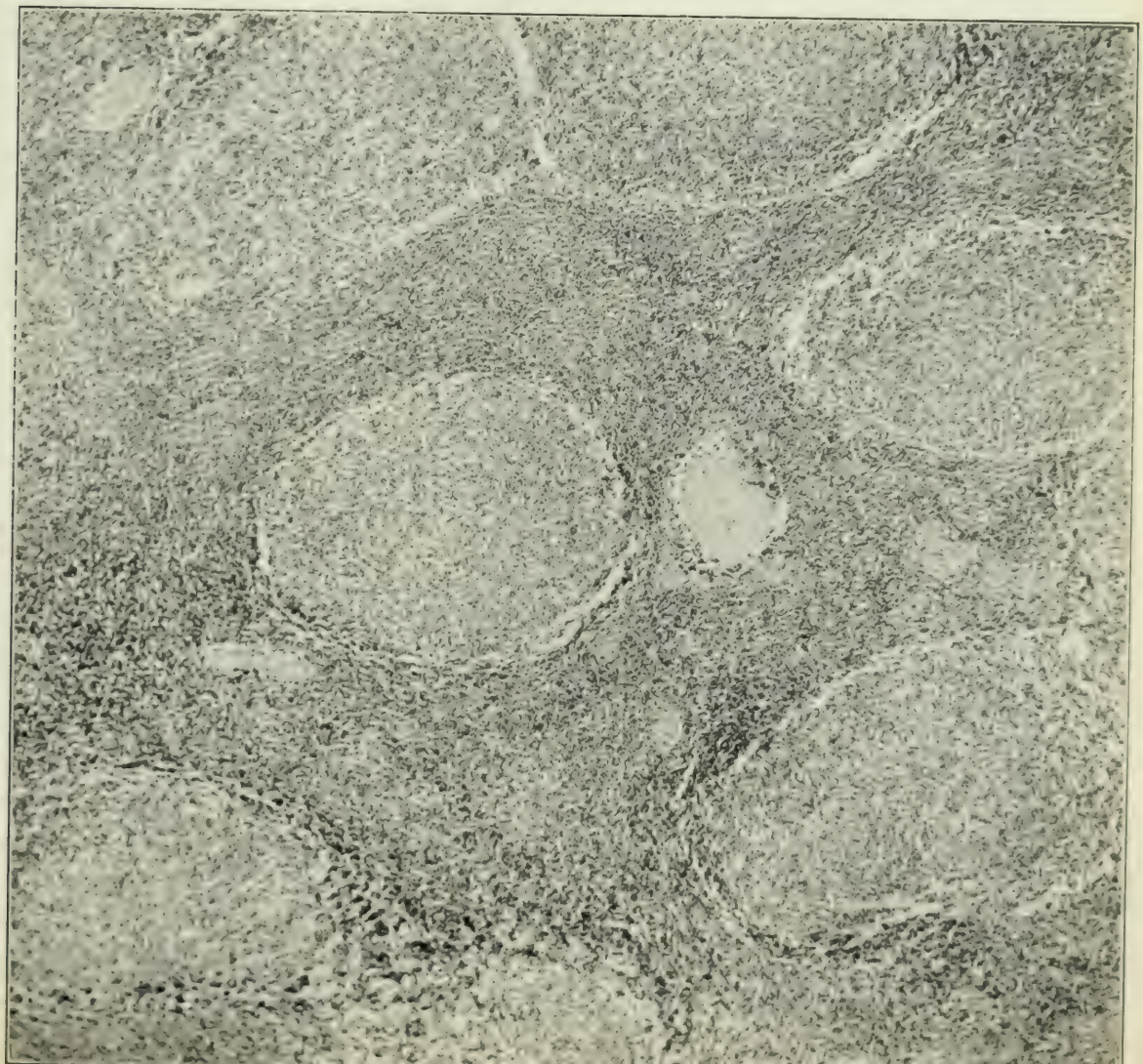


FIG. 11.



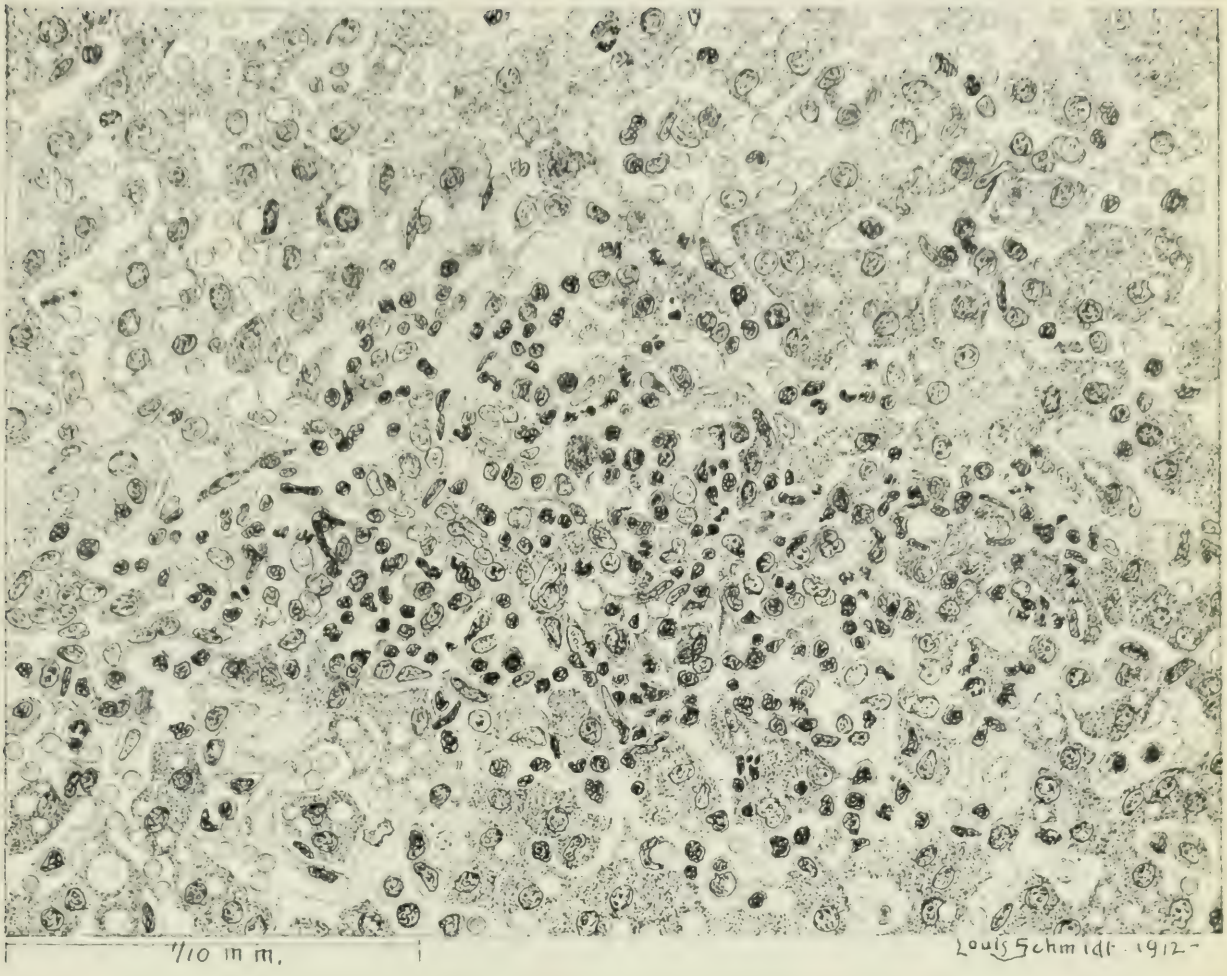


FIG. 13.

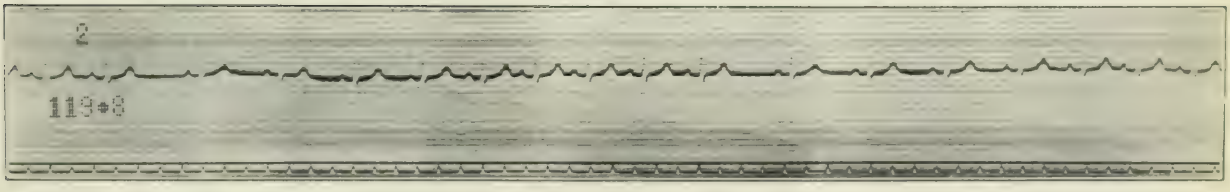


FIG. 14.

FIG. 15.

FIG. 16.

FIG. 17.

FIG. 19.

FIG. 18.

FIG. 20.

FIG. 21.

FIG. 22.

MD-2D

Reprinted from The Journal of Biological Chemistry, Vol. XII, No. 1, 1912

PICROLONATES OF THE MONOAMINO-ACIDS.

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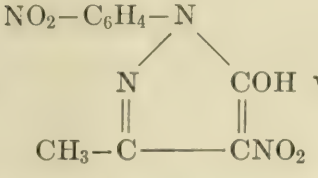
(Received for publication, May 29, 1912.)

Picrolonic acid  was first introduced by

Knorr[1] as a precipitant for organic bases. Steudel[2] applied it to the hexone bases obtained by hydrolysis of proteins. Later Mayeda[3] described also the picrolonates of the aromatic amino-acids, tryptophane and phenylalanine. The latter was particularly distinguished by its slight solubility in water. In the course of a hydrolysis we found this picrolonate of so much assistance in the isolation of phenylalanine that we attempted to make the picrolonates of some of the other monoamino-acids, with the idea that they also might prove useful in the separations that are necessary in carrying out protein hydrolyses. We found that all the natural monoamino-acids, with the exception of the pyrrollidine acids, proline, and oxyproline, gave beautiful crystalline, definite salts with picrolonic acid, most of them being fairly insoluble in cold water. When this work had been completed and the report sent in to the Secretary of the Society for Experimental Biology and Medicine for the meeting of May 15, an article by Abderhalden and Weil[4] appeared describing picrolonates of alanine, glycocoll, and inactive leucine. This

[1] *Ber. d. deutsch. chem. Gesellsch.*, xxx, p. 909, 1897.
[2] *Zeitschr. f. physiol. Chem.*, xxxvii, p. 219.
[3] *Ibid.*, li, p. 261.
[4] *Ibid.*, lxxviii, p. 150.

paper antedates our report in demonstrating that the ordinary monoamino-acids form crystalline picrolonates. As, however, our technique was somewhat different from that of Abderhalden and Weil, yielded products of constant molecular composition, and our work included the preparation and study of a larger series of picrolonates, we decided to publish it in the present paper.

Abderhalden and Weil added an alcoholic solution of picrolonic acid to the concentrated water solution of the amino-acids. Crystalline products were obtained, but, in the case of glycocoll and alanine, when an excess of the amino-acid was present the product contained more than 1 molecule of amino-acid. We never met this difficulty. Our procedure is as follows: The amino-acid and picrolonic acid in molecular proportions, or with amino-acid in excess, are dissolved in a minimum amount of boiling water. On cooling, and usually while the solution is still warm, the picrolonate crystallizes. The product obtained was of normal composition, containing one molecule each of amino-acid and picrolonic acid, in every case except those of *d*-alanine, *dl*-serine, and *d*-glutaminic acid. These salts showed a tendency to carry down an excess of picrolonic acid. The latter, however, was readily removed by shaking out the pulverized salt with ether. The salts all show definite crystalline form, which in some cases is quite characteristic. Isoleucine, for example, crystallizes in long slender prisms, while *l*-leucine forms rhomboids. If one makes a picrolonate of the so-called natural leucine from proteins, which is a mixture of the two, the two types of crystals can be seen together under the microscope. Most of the picrolonates decompose when they melt, and many of them have decomposing points too indefinite to be of value for characterizing them. Others, however, melt or decompose quite sharply.

For separating mixtures of amino-acids by means of their picrolonates two methods are possible. One can either transform all the amino-acids into picrolonates, and recrystallize them, or one can add to the mixture of amino-acids only enough picrolonic acid to combine with that forming the most insoluble picrolonate. We have not tried out either method exhaustively but we have found that the latter gives good results in separating phenylalanine from glutaminic and aspartic acids.

For determining the molecular proportions in which amino-acid and picrolonic acid are present in a given salt the most direct method is determination of the proportion of amino nitrogen present. The determination is conveniently carried out as follows: 0.1 — 0.2 gram of picrolonate is dissolved in boiling water to which 1 cc. of 10 per cent hydrochloric acid is added. The solution is cooled, and the picrolonic acid which crystallizes out is filtered off, the filtrate being received into an evaporating dish. The crystals are washed a few times, and the filtrate concentrated on the water bath, and used for determination of amino nitrogen by the nitrous acid method of Van Slyke.[5] The removal of picrolonic acid is necessary, because, even when the solubility of the picrolonate permits using it directly for the determination, the picrolonic acid set free in the nitrous acid mixture makes a gelatinous mass which renders the determination difficult.

The free amino-acids can be readily and quantitatively regained from the picrolonates by boiling the latter in water containing an excess of $\frac{N}{1}$ sulphuric acid, filtering off the picrolonic acid which crystallizes from the cooled solution, and extracting with ether the small amount of picrolonic acid remaining in solution. The solution is heated on the water bath, and the sulphuric acid precipitated with an equivalent of titrated barium hydrate solution. On evaporating the filtrate from the barium sulphate the free amino-acid is left in pure condition as a residue.

In the following table is given a list of the picrolonates which we have prepared, with some of the more important constants of each. The picrolonates are arranged in inverse order of their solubility.

[5] This *Journal:* ix, p. 185, 1911.

TABLE 1.

PICROLONATE OF	EMPIRICAL FORMULA	MOLECULAR WEIGHT	PERCENTAGE OF AMINO-ACID IN PICROLONATE	GRAMS SOLUBLE IN 100 cc. H_2O AT 20–23°	AMINO-ACID IN PICROLONATE DISSOLVED BY 100 cc. H_2O	MELTING OR DECOMPOSITION POINT (UNCORR.)	SPECIFIC ROTATION $[\alpha]_D^{20}$
dl-Phenylalanine	$C_{19}H_{19}N_5O_7$	429.2	38.4	0.12	0.05	212° (decomp.)	–
Tyrosine	$C_{19}H_{19}N_5O_8$	445.2	40.7	0.29	0.12	Blackens 260°	–
l-Phenylalanine*	$C_{19}H_{19}N_5O_7$	429.2	38.4	0.34	0.13	208° (decomp.)	+ 30.1°
dl-Leucine	$C_{16}H_{21}N_5O_7$	395.2	33.1	0.53	0.17	Indefinite over 140°	–
l-Leucine	$C_{16}H_{21}N_5O_7$	395.2	33.1	0.55	0.18	Indefinite about 150°	+ 19.6°
d-Isoleucine	$C_{16}H_{21}N_5O_7$	395.2	33.1	0.58	0.19	170°	+ 32.8°
dl-Valine	$C_{15}H_{19}N_5O_7$	381.2	30.7	0.81	0.25	Indefinite over 150°	–
dl-Serine	$C_{13}H_{15}N_5O_8$	369.2	28.4	0.98	0.28	Decomp. 265°	–
Glycocoll	$C_{12}H_{13}N_5O_7$	339.2	22.1	0.99	0.22	214° (decomp.)	–
dl-Alanine	$C_{13}H_{15}N_5O_7$	353.2	25.2	1.01	0.25	216° (decomp.)	–
d-Valine*	$C_{15}H_{19}N_5O_8$	381.2	30.7	1.20	0.37	170°–180°	+ 29.2°
d-Alanine	$C_{13}H_{15}N_5O_8$	353.2	25.2	1.61	0.41	214° (decomp.)	+ 12.4°
dl-Aspartic acid	$C_{14}H_{15}N_5O_9$	397.2	33.5	1.69	0.57	Blackens 130°	–
dl-Glutaminic acid	$C_{15}H_{17}N_5O_9$	411.2	35.8	2.37	0.85	184° (decomp.)	–
d-Glutaminic acid	$C_{15}H_{17}N_5O_9$	411.2	35.8	easily sol.		184° (decomp.)	+ 8.5

* The phenylalanine available was 25 per cent racemicized, consequently solubility and melting point may be slightly different in the optically pure substance. The rotation is calculated for optically pure substance. The *d*-valine was 19.8 per cent racemic.

All rotations were taken with alcoholic solutions except that of *d*-glutaminic acid, of which aqueous solution was used.

EXPERIMENTAL.

dl-Alanine. One gram of alanine and 2.6 grams of picrolonic acid (0.9 molecule) were dissolved in 20 cc. of boiling water. The salt crystallized while the solution was still hot. The yield was 3.2 grams. The crystals were long slender prisms. They melt at 216° with decomposition.

ANALYSIS: 0.200 gram substance; 13.40 cc. nitrogen at 22°, 760 mm.

	Calculated:	Found:
NH_2-N	3.96	3.78

d-Alanine. 0.445 gram of *d*-alanine and 1.32 grams (1 molecule) of picrolonic acid were dissolved in 15 cc. of water. The substance crystallized while the solution was still warm, forming long slender prisms. Yield, 1.3 grams. Analysis showed only 3.10 per cent of amino nitrogen instead of the theoretical 3.96. The substance was pulverized and shaken up with ether. It now gave the following figures:

ANALYSIS: 0.200 gram substance: 13.70 cc. N at 20°, 760 mm.

	Calculated:	Found:
NH_2-N	3.96	3.90

The substance melts at 214° with decomposition.

ROTATION IN ALCOHOLIC SOLUTION: Substance, 0.0496 gram; solution, 0.6141 gram; concentration, 8.07 per cent; specific gravity, 0.82; rotation in 0.5 dm. tube = + 0.41°.

$$[\alpha]_D^{20} = + 12.4° \pm 0.3°$$

Abderhalden and Weil give 215° as the melting point, + 11.1° as the specific rotation.

dl-Aspartic acid. 0.67 gram of aspartic acid and 1.32 grams of picrolonic acid (1 molecule) were dissolved in 50 cc. of boiling water. The solution was cooled in the ice box and allowed to stand over night for the crystallization to become complete. The crystals were long slender prisms with square ends.
The substance blackens at 130°.

ANALYSIS: 0.100 gram substance; 6.10 cc. N at 20°, 764 mm.

	Calculated:	Found:
NH_2-N	3.53	3.49

dl-Glutaminic acid. 0.736 gram of glutaminic acid + 1.32 grams of picrolonic acid (1 molecule) were dissolved in 10 cc. of water. Crystallization began only after the solution had been cooled to room temperature and scratched. The first crystals came in tangled threads, the later ones formed in very fine short spindles or needles. Yield, 1.7 grams. The substance softens at 183° and decomposes at 184°. These points are sharply defined.

ANALYSIS: 0.200 gram substance; 11.8 cc. nitrogen at 20°, 762 mm.

	Calculated:	Found:
NH_2-N	3.40	3.36

d-Glutaminic acid. 0.74 gram of pure *d*-glutaminic acid and 1.32 grams of picrolonic acid (1 molecule) were dissolved in about 10 cc. of water. On cooling 0.7 gram of crystals separated. On analysis, however, they were found to contain about two molecules of picrolonic acid. They were shaken out with ether, and then gave more nearly correct figures on analysis (3.25 per cent NH_2-N). The filtrate from this first crop was cooled to 0°, and a second crop separated, which proved to be fairly pure without ether treatment. The substance, like the inactive salt, softens at 183° and melts at 184°. The rotation was taken in water solution.

ANALYSIS: 0.100 gram substance; 6.3 cc. N at 766 mm., 21°.

	Calculated:	Found:
NH_2-N	3.40	3.59

ROTATION: 0.0562 gram substance; solution, 2.683 grams; concentration, 2.10 per cent; specific gravity 1.01; rotation in 0.5 dm. tube = + 0.09°.

$$[\alpha]_D^{20} = +8.5° \pm 1°$$

Glycocoll. 0.375 gram glycocoll and 1.32 grams picrolonic acid (1 molecule) were dissolved in 15 cc. of boiling water. The salt began to crystallize in rhomboid prisms of very characteristic appearance while the solution was still warm. It was allowed to stand over night and then yielded 1.56 grams of the salt. It melts sharply at 214 to 215° with decomposition.

ANALYSIS: 0.200 gram substance; 15.8 cc. N at 20°, 762 mm.

	Calculated:	Found:
NH_2-N	4.12	4.19

Abderhalden found that in alcoholic solution in the presence of an excess of glycocoll a picrolonate was obtained containing two molecules of glycocoll to one of picrolonic acid. We repeated the experiment, using two molecules of glycocoll, but an aqueous solution. The product was a normal salt identical with the above. 0.75 gram of glycocoll and 1.32 gram of picrolonic acid were dissolved in 15 cc. of boiling water and cooled. Crystallization began while the solution was still warm, yielding the above described characteristic rhomboids. Yield, 1.54 grams. Melting point, 214–215°.

ANALYSIS: 0.200 gram substance; 15.3 cc. N at 22°, 750 mm.

	Calculated:	Found:
NH_2-N	4.12	4.13

In calculating the nitrogen for both the above analyses the results are multiplied by the factor 0.93 in order to correct for the abnormal behavior of glycocoll with nitrous acid, which has been described in a previous paper by one of us.[6]

d-Isoleucine. 0.35 gram isoleucine, of + 37.4° specific rotation in 20 per cent hydrochloric acid, and 0.66 gram of picrolonic acid (0.9 molecule) were dissolved in 15 cc. of boiling water. While the solution was still warm the salt began to separate as drops which changed quickly to crystals. The latter were long, slender, six-sided and grouped in stars. The substance has a rather indefinite melting point at 170°, without decomposition.

ANALYSIS: 0.100 gram substance; 6.40 cc. N at 23°, 760 mm.

	Calculated:	Found:
NH_2-N	3.54	3.59

ROTATION: 0.0672 gram substance; solution in alcohol, 1.039 gram; concentration, 6.47 per cent; specific gravity, 0.82; rotation in 0.5 dm. tube = + 0.87°.

$$[\alpha]_D^{20} = + 32.8° \pm 0.3°$$

l-Leucine. 0.66 gram of *l*-leucine, of + 15.2° specific rotation in 20 per cent hydrochloric acid, was dissolved with 1.32 grams (1 molecule) of picrolonic acid in 15 cc. of boiling water. While the solution was still hot the salt began to settle out as an oil,

[6] Van Slyke: This *Journal*, ix, p. 185, 1911.

which crystallized at once when stirred with a rod. Yield, 1.7 grams. The substance begins to soften at about 145° and melts completely at 150° with evolution of a few small gas bubbles.

ANALYSIS: 0.200 gram substance; 12.4 cc. N at 22°, 750 mm.

	Calculated:	Found:
NH_2-N	3.54	3.45

ROTATION: 0.0462 gram substance; solution in alcohol, 0.6390 gram; concentration, 7.23 per cent; specific gravity, 0.813; rotation in 0.5 dm. tube = + 0.58°.

$$[\alpha]_D^{20} = + 19.6° \pm 0.3°$$

The salt forms beautiful rhomboid crystals, markedly different from the long slender ones of isoleucine picrolonate.

"Natural" Leucine. The leucine used was analytically pure, but of + 23° specific rotation in 20 per cent hydrochloric acid, and was a typical sample of the mixture of leucine and isoleucine usually obtained from hydrolyzed proteins. 0.6 gram of this leucine and 1.32 grams picrolonic acid were dissolved in 20 cc. of boiling water. While the solution was still warm the salt began to separate out in oil drops which quickly became crystalline when stirred. Under the microscope the long slender crystals of isoleucine picrolonate were seen mingled with the rhomboid ones of *l*-leucine picrolonate. The substance melts at about 170° without marked decomposition.

ANALYSIS: 0.2298 gram substance; 14.9 cc. N at 19°, 758 mm.
0.1275 gram substance; 0.2280 gram CO_2; 0.0623 gram H_2O.

	Calculated:	Found:
C	48.58	48.70
H	5.29	5.35
NH_2N	3.55	3.55

dl-Leucine. 1.4 grams of leucine and 2.64 grams of picrolonic acid were dissolved in 30 cc. of boiling water. The salt separated as an oil while the solution was still warm, but crystallized quickly in six-sided prisms. The last crystals separating formed rosettes with spurs a centimeter long. The melting point is very indefinite, and dependent upon the manner in which the substance is heated. When plunged at once into a bath at 150° or higher

it decomposes instantly, but when heated from 130° upwards it slowly sinters and may not be decomposed until it reaches 188°.

ANALYSIS: 0.200 gram substance; 12.20 cc. N at 20°, 760 mm.

	Calculated:	Found:
NH_2-N	3.54	3.47

l-Phenylalanine Picrolonate. One gram of phenylalanine, of rotation $-26.7°$ in water solution, was dissolved with 1.6 grams of picrolonic acid in boiling water. The salt crystallized while the water was still hot. Under the microscope two types of crystals could be seen, long slender rods clustered in stars, and short rectangular prisms separate or in small aggregates. When the substance was filtered it was seen to consist of an apparent mixture of yellow and orange crystals. The non-homogeneous appearance, however, was merely due to the presence of two types of crystals. The substance melted at 208° with decomposition, gave theoretical results for carbon and hydrogen on combustion, and the following figures for amino nitrogen.

ANALYSIS: 0.2148 gram substance; 12.3 cc. N at 20°, 762 mm.

	Calculated:	Found:
NH_2-N	3.26	3.27

ROTATION: 0.0696 gram substance; solution in alcohol, 1.080 gram; concentration, 6.44 per cent; specific gravity, 0.818; rotation in 0.5 dm. tube $= +0.60°$.

$$[\alpha]_D^{20} = +22.8° \pm 0.3°$$

In order to ascertain whether the picrolonate contained a proportion of the optically active phenylalanine different from that of the partially racemicized substance from which it was made, a portion of the picrolonate was freed from picrolonic acid and the pure phenylalanine regained as described in the introduction. It showed a specific rotation of $-26.7°$, and was therefore identical with that used at the beginning. The rotation of optically pure *l*-phenylalanine being $-35.3°$ this sample was evidently 25 per cent racemic and 75 per cent active. Dividing the rotation of the picrolonate by 0.75 gives 30.1° as the specific rotation of the picrolonate of pure active *l*-phenylalanine.

dl-Phenylalanine. 0.83 gram of phenylalanine and 1.32 grams (1 molecule) of picrolonic acid were boiled with 40 cc. of water. The salt was so insoluble that complete solution could not be obtained, even in this volume of water. The product was, however, pure. The yield was practically quantitative, 2.04 grams. The substance decomposes quite sharply at 211 to 212°. Examined under the microscope it is seen to consist of only the short rectangular type of prisms.

Analysis: 0.1000 gram substance; 5.80 cc. N at 20°, 764 mm.

	Calculated:	Found:
NH_2-N	3.26	3.32

dl-Serine. 0.26 gram of serine and 0.66 gram of picrolonic acid were dissolved in 5 to 10 cc. of water. The salt crystallized in long slender rods while the solution was still warm. It contained, however, an excess of picrolonic acid, as was shown by its low content (2.56 per cent) of amino nitrogen. A second preparation showed identical composition, 2.59 per cent amino nitrogen. The melting point was indefinite at 130 to 140°. After the substance had been shaken out thoroughly with ether, it could be heated to 200° without change. Above 200° it began slowly to darken, and finally decomposed with foaming at 265°. It now gave the following figures:

Analysis: 0.1300 gram substance; 7.9 cc. N at 23°, 764 mm.

	Calculated:	Found:
NH_2-N	3.79	3.43

Tyrosine. 0.91 gram of tyrosine and 1.32 grams of picrolonic acid (1 molecule) were dissolved in 40 cc. of boiling water. On cooling the salt crystallized at once in rods grouped together in rosettes. The substance blackens and sinters at 260° without melting or foaming.

Analysis: 0.200 gram substance; 10.70 cc. N at 22°, 760 mm.

	Calculated:	Found:
NH_2-N	3.14	3.02

d-Valine. 1.3 grams of valine and 2.64 grams of picrolonic acid (0.9 molecule) were dissolved in 10 cc. of boiling water. The

salt showed a tendency to separate as an oil, which, however, changed to crystals while the solution was still warm. Yield 3.4 grams. The substance melts between 170° and 180°, without decomposition. The melting point is not sharply defined.

ANALYSIS: 0.200 gram substance; 12.95 cc. N at 23°, 760 mm.

	Calculated:	Found:
NH_2-N	3.67	3.63

ROTATION: 0.0471 gram substance; solution in alcohol, 0.834 gram; concentration, 5.62 per cent; specific gravity, 0.82; rotation in 0.5 dm. tube = + 0.54°.

$$[\alpha]_D^{20} = + 23.4° \pm 0.2°$$

The free valine was regained from 0.5 gram of the picrolonate and its rotation determined: substance, 0.1166 gram; solution in 20 per cent hydrochloric acid, 2.782 grams; concentration, 4.19 per cent; specific gravity, 1.10; rotation in 2 dm. tube = + 2.13°.

$$[\alpha]_D^{20} = + 23.1° \pm 0.1°$$

The valine from which the picrolonate was made had been obtained from a protein hydrolysis and showed practically the same rotation. Taking + 28.8° as a rotation of optically pure *d*-valine, the sample used is calculated to contain 19.8 per cent of racemic valine and 80.2 per cent of the active. Dividing the rotation observed for the picrolonate by 0.802 gives + 29.2° as the rotation of the picrolonate of the pure *d*-valine.

dl-Valine. 0.60 gram of Kahlbaum's valine and 1.32 grams of picrolonic acid (1 molecule) were dissolved in 15 cc. of boiling water. Crystallization began while the solution was still warm. Yield, 1.84 grams. The substance melts at once with vigorous decomposition if plunged into a bath of 150°; if heated from below 130° upwards it gradually darkens and sinters, and may not decompose until it reaches 220°.

ANALYSIS: 0.200 gram substance; 13.0 cc. N at 21°, 754 mm.

	Calculated:	Found:
NH_2-N	3.67	3.65

Solubility Determinations.

For the determination of the solubility in water, 0.4 gram of picrolonate, finely ground, was shaken 4–5 hours with 15 cc. of water at room temperature, which varied between 20 and 23°. The solutions were filtered, and 10 cc. portions of the filtrate evaporated to dryness in weighed glass dishes. The results are given in Table 1.

For the determination in alcohol the same technique was employed, except that only 8 cc. of the solvent were used and only 5 cc. evaporated. The following picrolonates are readily soluble in cold alcohol: those of *d*-alanine, *d*-isoleucine, *l*-leucine, *dl*-leucine, tyrosine, and *l*-phenylalanine. *d*-Valine and *dl*-valine dissolve readily when alcohol is warmed slightly. The following solubilities in absolute alcohol were determined quantitatively:

TABLE 2.

PICROLONATE OF	GRAMS SOLUBLE IN 100 CC. ALCOHOL AT 20° TO 23°
dl-Phenylalanine	0.81
dl-Aspartic acid	1.16
dl-Alanine	2.88
dl-Glutaminic acid	3.86
l-Phenylalanine	5.71

The *l*-phenylalanine used was that previously described, and contained 25 per cent of the racemic substance. Although the figures cannot, therefore, be regarded as strictly accurate, they show that the picrolonate of *l*-phenylalanine is several times more soluble in absolute alcohol than that of the *dl*-phenylalanine. By utilizing this difference in solubility *l*- and *dl*-phenylalanine can be partially separated. A mixture of three parts active and five parts inactive picrolonate was shaken with twenty parts of alcohol. Half of the substance was dissolved. Its rotation showed that it was 75 per cent active. The residue was absolutely inactive.

Separation of Phenylalanine and Aspartic Acid.

In 20 cc. of boiling water were dissolved 0.75 gram of phenylalanine (specific rotation −27°), 1.25 grams (1 molecule) picro-

lonic acid, and 0.7 gram of *dl*-aspartic acid. The solution was cooled and allowed to stand two hours for crystallization to become complete. The picrolonate was then filtered off and washed with cold water. It weighed 1.76 grams, equivalent to 0.678 gram of phenylalanine. Analysis showed it to be the pure phenylalanine picrolonate.

ANALYSIS: 0.1354 gram substance; CO_2, 0.2645 gram; H_2O, 0.0555 gram.

	Calculated:	Found:
C	53.12	53.13
H	4.46	4.63

In a similar manner we were able to separate a mixture of glutaminic acid and phenylalanine which we encountered in a hydrolysis of hemoglobin that will shortly be the subject of another article. The use of picrolonic acid in the isolation of phenylalanine by the ester method decidedly improves the accuracy of the determination. Because of the crystalline nature of the picrolonates and the insolubility of many of them, it is possible that with their assistance the number of amino-acids which can be obtained from hydrolyzed proteins without esterification may be increased.

THE PRESERVATION OF TISSUES AND ITS APPLICATIONS IN SURGERY*

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I. INTRODUCTION

Six years ago, at the Rockefeller Institute, I began to investigate how tissues isolated from the organism could be preserved and used after a few days or weeks as grafts. I wished to find a method by which tissues, extirpated from the amputated limb of a living animal or a fresh cadaver, could be stored during the period which elapses between their extirpation and their transplantation on the patient. It would be very convenient for the surgeon to keep in store pieces of skin, periosteum, bone, cartilage, blood-vessels, peritoneum, omentum and fat, ready to be used.

I attempted to preserve the tissues outside of the organism in a condition of latent or active life.

A tissue is in latent life when its metabolism becomes so slight that it can hardly be detected, and also when its metabolism is completely suspended. Latent life means, therefore, two different conditions — unmanifested actual life and potential life. Unmanifested actual life is a normal stage of the evolution of all organisms, when they progress from general death toward elemental death. As the metabolism is still going on, although very slowly, it is a temporary condition. Sooner or later cadaveric lesions develop which bring about the complete disintegration of the protoplasm. Potential life consists of a suspension of all actual vital processes. The metabolism being suppressed, no cadaveric changes would take place in the protoplasm. Tissues in a condition of potential life could be preserved outside of the body for an indefinite period of time.

* Read in the Section on Surgery of the American Medical Association at the Sixty-Third Annual Session, held at Atlantic City, June, 1912.

* From the Rockefeller Institute for Medical Research.

Latent life was discovered two centuries ago by Loewenhoeck, who obtained the resurrection of *Milnesium tardigradum*, which had been completely dried for a long time, by moistening it with water. In 1840, Doyere also studied the peculiarities of latent life of *Milnesium tardigradum*. He completely dried a few of these animals, heated them at a temperature of 100 C., and, after having humidified them, observed that they lived again. These observations are very important because *Milnesium tardigradum* is highly organized and contains muscular fibers, nerves, nervous ganglia, etc. Paul Bert, in several famous experiments, preserved in latent life the tissues of mammals, and succeeded in transplanting rats' tails, kept for several days in a small quantity of confined air at a temperature not higher than + 12 C. In 1907-08 I transplanted successfully vessels preserved for several days or weeks in cold storage in a condition of unmanifested actual life.

A tissue isolated from the organism can be kept in active life if it is given artificially a normal nutrition. The possibility of the active life of a tissue outside of the organism was demonstrated in 1907 by Harrison, in a series of splendid experiments made in the anatomic laboratory of Johns Hopkins University. Harrison transplanted the central nervous system of frog embryo in a drop of fluid taken from the lymph-sac of an adult frog. He could then observe, during a few days, the growth of the axis cylinders. In 1910, with the collaboration of Dr. Burrows, I observed the growth of tissues of mammals in plasma. In 1912, I found that the permanent active life of the tissues outside of the organism was possible: a piece of a chick's heart pulsated strongly one hundred and four days after its extirpation, and connective tissue was growing actively during the fifth month which followed its extirpation from the organism.

The discovery of Loewenhoeck, Paul Bert and Harrison of these two forms of life—latent life and active life—of the tissues separated from the organism, was of great biologic and philosophic importance, but it did not seem that it would ever be of practical interest. Nevertheless, it appears to-day that the evolution of surgery may lead to the use in human therapeutics of the work of these great biologists.

II. EXPERIMENTS

From 1906 to 1912 several technics were developed in order to keep tissues isolated from the organism in a condition of latent or active life.

These tissues were extirpated from a living animal or from an animal recently killed by chloroform. The death of the whole organism does not bring about immediately elemental death, that is, death of the tissues and organs. Elemental death is a slow process, while general death may occur suddenly. For instance, a man is stabbed through the heart and killed. His personality has disappeared. He is dead in the medicolegal sense of the word. All the organs and tissues which compose the body, however, are still living. If it were possible immediately after death to transplant the tissues and organs which compose this body into other organisms of identical character, no elemental death would occur, and all the constituent parts of the body would continue to live. In this case, general death can be defined as the rupture of the contract of association between the tissues and organs of the organism by failure of one of the partners, the heart. Therefore, general death is merely the starting-point of the disintegrative phenomena which lead to elemental death, that is, destruction of the living matter.

The duration of the period which elapses between general and elemental death varies according to the nature of the tissues. The cerebral substance disintegrates so quickly that, after a few minutes of complete anemia, irreparable lesions take place, while skin, bones and connective tissues can overcome for a long time the cadaveric process. Therefore it was necessary to extirpate, soon after death, such glands as the hypophysis and thyroid, which disintegrate rapidly, while the more resistant tissues, like the skin and arteries, could be removed from the animal a few hours after death. The tissues must be extirpated before the beginning of the cadaveric disintegration. It is well known that elemental death is brought about by microbic and autolytic enzymes. Immediately after general death, the microorganisms from the digestive tract diffuse through the body and their ferments begin to destroy the tissues. At the same time, the autolytic ferments, which are no longer held in check by the serum, contribute also to the disintegration of the organs. This destructive process

is increased or retarded by the causes which activate or retard the enzymotic actions, and the multiplication of the microorganisms. For instance, the rate of cadaveric disintegration, which is very rapid at 35 or 40 C., becomes very slow at — 1 or — 2 C. It is, therefore, important immediately to put the cadaver from which the tissues will be used as grafts into cold storage, and to extirpate the tissues under rigid asepsis as soon as possible after death.

The experiments were performed on various tissues: blood-vessels, cartilage, periosteum, skin, spleen, heart, thyroid and hypophysis of adult dogs and of chick fetuses.

A. PRESERVATION OF THE TISSUES IN LATENT LIFE

Experiment 1.—The tissues were deposited in a preservative medium and put in a refrigerator immediately after their removal from the organism.

Several preservative mediums were used: isotonic sodium, chlorid solution, Locke's solution, Ringer's solution, defibrinated blood, serum, confined human air, and petrolatum. The vessel or the flaps of skin and periosteum were placed in tubes filled with the medium. Petrolatum and Ringer's solution were chiefly employed. The tubes were sealed or hermetically closed and deposited in a refrigerator, the temperature of which was maintained constantly between — 1 C. and + 7 C.

The color and consistency of the tissues remained generally normal for several weeks. After six, seven and even ten months the microscopic appearance of the arteries was not markedly modified. The wall was a little softer, and the vessel flattened more readily. A section of a pig's carotid artery, preserved in a sealed tube with a few drops of Locke's solution from April to November, 1908, looked as if it had been extirpated from the animal only a few moments since, and its histologic appearance was entirely normal. While the morphology of the blood-vessels, skin, cartilage, etc., preserved for a few months in cold storage was not modified, glands, such as the thyroid or kidney, showed, after a short time, a marked disintegration.

The appearance of a tissue does not give any indication of its dynamic condition. There is no morphologic difference between a living tissue and a dead tissue. The only method of finding out the condition of a tissue which is supposed to be in latent life is to replace it under its normal physiochemical conditions and to observe whether it will again manifest life.

The condition of the tissues preserved in cold storage was examined by two methods: cultivation and transplantation.

1. Fragments of spleen, skin and heart were extirpated from 15- or 16-day-old-chick embryos and preserved in Ringer's solution and in cold storage for one, two and even six days. Afterward they were put in chicken plasma and in an incubator. They grew luxuriantly. It was then demonstrated that tissues preserved in cold storage were really in a condition of latent life, since they resumed their growth as soon as they were given back their normal physiochemical conditions.

2. In a great many experiments, tissues which had been kept in cold storage from one day to eleven months were transplanted into animals and the results examined from a few days to four years after the operation.

In several experiments, flaps of dog's skin, preserved in petrolatum and in cold storage from one to ten days, were transplanted. Generally the graft took as well as a fresh piece of skin. In one case a fragment of black skin was grafted on a white dog. As the black hair grew from the skin no mistake was possible regarding the condition of the graft during the period of preservation in cold storage.

In a few experiments, pieces of dog's cartilage and periosteum which had been kept in cold storage from twenty-four to twenty-eight hours were transplanted on dogs. It was found that periosteum produced bone and that cartilage remained alive.

In many experiments segments of dog's arteries and veins preserved in cold storage were transplanted on the carotid artery and on the abdominal or thoracic aortas of other dogs. The results were examined from five days to more than four years after the operation. The gross anatomic changes of the vessels were light, but the wall often presented marked histologic lesions. They consisted generally in the partial or complete disappearance of the muscular fibers. The remote results of these operations were excellent. For example, a segment of popliteal artery, extirpated from the leg of a young man and preserved for twenty-four days in cold storage, was transplanted onto the abdominal aorta of a small bitch. The animal lived in excellent health for four years and two months, became pregnant several times, and finally died during labor. The abdominal aorta was normal, but

the human arterial segment was slightly dilated, and its wall was composed of connective tissue only.

In another experiment a segment of a dog's jugular vein, preserved for twenty-four hours in cold storage, was transplanted onto the thoracic aorta of a fox terrier. In spite of slight lesions of the cord, due to interruption of the aortic circulation during the operation, the animal remained in excellent health. After two years and two months the dog died of an epidemic disease. The descending aorta was normal and the transplanted segment had about the same caliber as that of the aorta.

The results of these and many other experiments showed that tissues like blood-vessels, skin and connective tissue could be preserved in latent life for two weeks at least. When blood-vessels were kept in cold storage for several months, the results were often excellent, but it was impossible to ascertain whether the original tissue had not been replaced by the tissues of the host.

B. PRESERVATION OF TISSUES IN A CONDITION OF ACTIVE LIFE

Experiment 2.—Tissues cut into thin fragments and placed in a nutritive medium at the temperature of the body can be maintained in a condition of active life. In 1912, I attempted to preserve in that manner hypophysis and periosteum extirpated from dogs living or recently killed by chloroform. The best medium is normal plasma, as was demonstrated by a comparative study of the value of varied artificial and natural mediums made at the Rockefeller Institute by Ragnvald Ingebrigtsen. The plasma was prepared according to the technic of Delezenne and Bordet and Gengou. An oiled glass cannula was introduced into the carotid artery of a dog. The blood was allowed to flow into cold paraffined tubes, which were put in ice and centrifugalized. The plasma was then taken up with paraffined pipets, put into large glass tubes or spread on the surface of the covers of Gabritschewski boxes. The large periosteal flaps were dropped into the tubes, and the small fragments of hypophysis were immerged into the layer of plasma covering the covers of the boxes. Coagulation took place rapidly. Then the tubes and the boxes were sealed and placed in an incubator at a temperature of 38 C.

After a few days it was found that the fragments of hypophysis were living, and that continuous layers or tubular formations of epithelial-like cells had grown from their edges through the culture medium. Around small pieces of periosteum which were cultivated in hanging-drops at the same time as the large flaps were deposited in the tubes a new tissue had developed. The growth of the hypophysis stopped after about five or six days, and that of the periosteum after nine or ten

days. In order to preserve these tissues for a longer time it would have been necessary to wash and transplant them into a new medium.

Transplantation of the periosteum preserved in active life was made after one, two and three days. The periosteal flaps were removed from the plasma and washed in Ringer's solution. Afterward, they were placed under the skin of the chest of a dog. Periosteal flaps preserved in Ringer's solution were used as controls. The results were examined after about one month. It was found that more bone was produced by the flaps preserved in plasma than by the controls.

It is possible that a tissue, like periosteum, cartilage or skin, would grow better after having been preserved in active life than after having been kept in cold storage. Glandular tissues, like hypophysis or thyroid glands, which were not preserved successfully in cold storage, could live actively for five days at least in Gabritschewski boxes. Nevertheless, while the preservation of a tissue in active life is better than in latent life, it is also very much less safe.

The technic is more complicated and the danger of infection very much greater. The more active the metabolism of a tissue, the more sensitive are the cells to external influences. Therefore, for the preservation of tissues used at present in human surgery, the cold-storage method is simpler and more practical and must be used.

III. CLINICAL APPLICATION

It is known that flaps of human skin preserved for some time at a low temperature have already been used in human surgery. Nevertheless, the first systematic application of the preservation of tissues in latent life was made by Dr. Tuffier. In 1910 and 1911 he preserved in petrolatum and in a refrigerator pieces of fat, bone, cartilage and peritoneum which he had extirpated from amputated limbs. He also used lipomas and fragments of ovaries and of peritoneum which had to be extirpated during certain operations. These tissues were kept in cold storage from a few hours to two months. Grafts of lipoma and omentum were inserted by Dr. Tuffier between the pleura and the thoracic wall in several cases of pleurisy and intrapulmonary abscesses. In the reconstruction of joints, Dr. Tuffier several times used pieces of omentum, peritoneum and cartilage. In a case of resection of the elbow he covered the surface of the section of the bones with cartilage preserved for

five days in cold storage. After seventeen months the patient was examined and the result found excellent. In another case of resection of the elbow, Dr. Tuffier covered the ends of the bones with a fragment of omentum preserved for one month in cold storage. Ten days afterward the elbow-joint could be moved easily. In ten operations of the same or similar character, there was neither elimination of the graft nor other accidents.

In 1911 Dr. Magitot of Paris extirpated the eye of a patient suffering with glaucoma. The eye was placed in a tube containing human serum, and kept for eight days in a refrigerator at a temperature of +4C., during which period the cornea remained transparent. Dr. Magitot resected the anterior part of the cornea of a man who had been burned by alkali. A large scar had developed on the cornea and the patient was blind. In the opening, a flap of cornea from the eye preserved in cold storage was inserted. The graft took. Seven months after operation the transplanted cornea had remained transparent and the patient could see through it.

The results obtained by Dr. Tuffier and Dr. Magitot showed that tissues preserved in cold storage could be used in clinical surgery.

At the end of 1911 I attempted to develop a technic which would permit a large quantity of human tissues to be kept in store ready for transplantation. The tissues which are needed by surgeons in their clinical work are skin, bone, cartilage, periosteum, peritoneum, fat, tendons and aponeuroses. Some of these tissues could be extirpated from amputated limbs. I thought, however, that tissues of a better quality could be taken from the cadavers of the fetuses which had died during labor in the maternity hospitals. One cadaver, properly cut and preserved, would furnish enough tissues for many operations.

Dec. 6, 1911, the cadaver of an infant who had died during labor was used for the extirpation of cutaneous and other grafts. Several hours after death the body of the child was washed with soap and water and with ether. Dermo-epidemic grafts and flaps of skin were extirpated in large numbers and washed in Ringer's solution. Bones were also extirpated. Afterward the grafts were put in tubes containing warm petrolatum. Other grafts were preserved in Ringer's solution. The tubes were deposited in a refrigerator at + 3 C.

The Wassermann reaction made by Dr. Swift with the blood taken from the heart of the cadaver was negative.

The culture mediums inoculated with small fragments of the skin remained sterile.

The dermo-epidemic grafts and the flaps of skin preserved in petrolatum and in Ringer's solution were used at the Rockefeller Hospital for the treatment of three large ulcers and one circular ulcer of the legs of two patients.

The flaps of skin preserved in petrolatum remained apparently normal. Forty-two days after the death of the child the skin preserved in petrolatum was bluish, but when exposed to the air it became pink again. The small vessels of the subcutaneous tissue were filled with blood which was red and still fluid. Histologic sections of the skin showed that it was normal. At the end of May, 1912, the appearance of the flaps preserved in petrolatum was not markedly changed. Ringer's solution was not so good a preservative medium as petrolatum. After a few weeks the flaps of skin preserved in Ringer's solution began to disintegrate slightly.

We began to graft the flaps of skin on the ulcers of the patients after twenty-four hours. The flaps were taken from the tubes and put for a few minutes in Ringer's solution at the temperature of the body. Then they were removed from the solution, compressed between two pads of hydrophil gauze and applied on the granulations of the ulcers. They were rapidly fixed to the granulatory surface by a warm mixture of paraffin, wax and castor oil, analogous to "ambrine," invented a few years ago by Dr. Barthe de Sandford, the composition of which remained unknown. The mixture I used is composed of 18 gm. of paraffin melting at 52 C., 6 gm. of paraffin melting at 20 C., 2 gm. of beeswax, and 1 gm. of castor oil.

The warm and fluid mixture was applied with a brush on the surface of the graft. It became solidified very rapidly, but it was not adherent to the grafts or to the granulations, and it was possible to take it out every day. The wounds and the grafts were washed with oil and a new coat of the mixture was applied. Most of the grafts took, just as fresh grafts would have done. The last grafts were applied on the ulcers after seven weeks of preservation in cold storage, and a few of them took. The length of the period during which the flaps

of skin could be preserved in latent life was not determined. It seems that the skin preserved during two weeks took as well as normal skin. As a great many pieces of skin can be kept in petrolatum, repeated grafts on the granulating wound could be made as easily as ordinary dressings with gauze.

IV. CONCLUSIONS

The results obtained by Tuffier, Magitot and myself demonstrated that human tissues preserved in cold storage could be used in human surgery. Future investigators will show in what measure tissues of infants should be employed as grafts. The grafts could easily be taken in large quantities from the fresh cadavers of fetuses and infants, and preserved in petrolatum and in cold storage. A supply of tissues in latent life would be constantly ready for use and the tubes containing the tissues could even be sent, in small refrigerators of the type of vacuum bottles, to surgeons who would need them. It would simplify very much the transplantations of skin, bone, periosteum and aponeuroses, which are more and more used in human surgery.

Reprinted from The Journal of the American Medical Association
August 17, 1912, Vol. LIX, pp. 523-526

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American Medical Association, 535 Dearborn Ave., Chicago

No. 22

SOME ASPECTS OF ANAPHYLAXIS

By John Auer, M.D.

[Reprinted from The Popular Science Monthly, November, 1912.]

SOME ASPECTS OF ANAPHYLAXIS

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THE word anaphylaxis is used to designate the train of symptoms and signs which is produced by the incorporation of a foreign soluble proteid into an animal organism which has already been subjected before to the action of this same foreign proteid. The first injection need cause no obvious disturbance at all, and the injected animal seems to be perfectly normal. But if this animal be reinjected after an appropriate interval, it will answer with marked reactions, which may even end in death. Thus, for example, 5 to 6 cubic centimeters of horse serum injected intraperitoneally in a guinea-pig cause no more apparent disturbance than the same quantity of physiological salt solution; but if the same animal receive the same amount of the same horse serum intraperitoneally after two or three weeks, the animal usually dies in a short time. The first injection, therefore, though it caused no obvious change in the animal, has profoundly altered its constitution, and it reacts on second injection as if the original substance were now a violent poison. The animal, however, does not acquire this remarkable property at once; approximately ten to fourteen days must elapse before the second injection elicits marked toxic effects. If the injection is repeated earlier, slight or no symptoms will be produced. It is thus clear that the organism requires a certain length of time before the second injection can call forth toxic symptoms. The whole process, then, shows three distinct phases:

(1) *Sensitization,* caused by the first injection of the foreign proteid; (2) *Incubation,* the time which elapses before the second injection can cause a response; and (3) the state of *Intoxication* which this second injection causes when given to a sensitized animal.

These three stages show some interesting points which deserve to be mentioned more in detail.

Sensitization.—Any soluble proteid may be used to sensitize an animal, provided that it is of foreign nature; nor need these proteids be of animal origin; Wells has recently shown that a large number of plant proteins may be used for this purpose.

The proteid usually employed in laboratory investigation, for anaphylaxis can only be studied by animal experiment, is horse serum, and horse serum is used only because it is easily obtainable, and is not poisonous to the ordinary laboratory animals on first injection. A

normal guinea-pig will easily tolerate five, a rabbit twenty and a dog one hundred cubic centimeters intravenously, without showing any obvious effect on blood-pressure or respiration. Harmlessness on first injection is, however, not an absolute essential, and animals may easily be sensitized by primarily toxic sera or poisonous animal extracts, for the amount needed to sensitize is very slight, and is only a small fraction of the lethal dose.

The amount necessary to sensitize is almost unbelievably minute; according to Rosenau and Anderson, 0.000,001 cubic centimeters of horse serum may suffice for a guinea-pig, and Wells has succeeded in sensitizing the same animal species with a still smaller quantity of pure egg albumen, 0.000,000,05 gram. These quantities are beyond the capacity of any balance or test tube to detect, and the biological reaction, as usual, is shown to be the most delicate.

The substance used for sensitization may be incorporated in a variety of ways: by subcutaneous, peritoneal or intravenous injection. Even by feeding the proteid, sensitization may be produced in the guinea-pig according to Rosenau and Anderson. The usual method employed, however, is either subcutaneous or intraperitoneal injection; both these procedures are swiftly and easily carried out, and give but slight or no discomfort to the animal.

Although not every species of animal has been tested, it seems probable that all may be sensitized. The only difference noted is that some species are more difficult to sensitize than others; the guinea-pig is most easily sensitized of all animals tested so far, and for this reason has been the classical animal for investigation. The dog and the rabbit are also rendered hypersusceptible with comparative ease. Fowl are more refractory; man also can be sensitized.

The length of time that sensitization lasts varies in the different animal species. In the guinea-pig that state persists for life, which is about three years (Rosenau and Anderson). In the rabbit the degree of sensitization diminishes after three or four weeks, but persists to a greater or less extent for many months, and in man symptoms have been noted seven years after the first injection.

The degree of sensitization varies also in the different animals and will be considered more fully later.

Incubation.—After the animal has received an unaltered foreign proteid into its circulating juices, this foreign material causes a profound change in the reactions of the host to this proteid. This change occurs gradually and reaches its maximum only after some weeks. If the animal is tested after a few days no reaction will be obtained. In guinea-pigs, for example, ten to fourteen days must elapse before an anaphylactic response can be expected with some certainty, and even with these animals it is best to allow three weeks to pass before testing.

The period of incubation, however, may be shortened if an animal is rendered *passively anaphylactic*. This process depends upon the fundamental observation of Gay and Southard that a normal guinea-pig may be sensitized by injecting it with the serum of another guinea-pig which is already sensitized. If a normal guinea-pig is thus injected with the serum of an animal (guinea-pig, or rabbit more usually) which was sensitized some weeks previously, this normal guinea-pig becomes fully sensitized within twenty-four hours and will respond with typical symptoms when injected with the same proteid which was used to sensitize the donor of the serum (Otto). The serum of an actively sensitized animal, that is, one sensitized by the injection of a foreign proteid, therefore contains some substance, termed a serum-rest or anaphylactin by Gay and Southard, which upon injection fully sensitizes a normal animal within a few hours.

Intoxication.—In this stage we observe how a sensitized animal responds with violent symptoms to an injection of the same proteid which it formerly tolerated with no apparent ill effect; we see the remarkable transformation of what formerly was an apparently harmless substance into a violent poison. The symptoms and signs noticeable in an animal during this stage vary with the species and with the site of injection of the toxic dose. If the injection is given subcutaneously in rabbits, an area of edema develops in the place injected; this edema may gradually lead to a circumscribed necrosis of the skin (phenomenon of Arthus). The same change may also occur in guinea-pigs, as Lewis has shown. If the second injection is given intravenously in rabbits, a more or less marked respiratory disturbance associated with muscular weakness and increased peristalsis develops (Arthus); if the rabbits are highly sensitized, convulsions followed by death occur in a few minutes (Arthus). In the dog, the respiratory symptoms are not prominent, but the animal shows nausea and vomiting, profound muscular weakness and often discharge of urine and feces. The animals, however, usually recover. In the guinea-pig, the stage of intoxication is dominated by respiratory symptoms. The animal makes such powerful respiratory attempts that the costal arch is drawn inwards with each inspiration; these efforts swiftly become convulsive and the animal dies a few minutes after the intravenous injection of an adequate dose (Auer and Lewis).

Anatomical and Functional Changes Found in the Stage of Intoxication.—The study of anaphylaxis from the clinical symptoms alone is unsatisfactory. The symptoms offer nothing which could not be produced by numerous drugs available to the investigator; they do not indicate *why* the animal shows these disturbances. For an adequate picture of the process the seat of these reactions and a finer analysis of the functional disturbances is necessary. Moreover, no rational thera-

peutic intervention is possible if the investigator is in the dark concerning these points. The first attempt to study anaphylaxis more thoroughly was made by Arthus in 1903. This investigator showed for the first time that anaphylaxis in rabbits is characterized by a marked drop in blood pressure. This drop in blood pressure Arthus considers the most delicate indicator of anaphylaxis. In 1910 Cesaris-Demel described the effects which were produced when the *excised* heart of a sensitized rabbit was perfused with a dilute solution of the same proteid which caused sensitization. He stated that such a heart rapidly decreased the amplitude of its beat and assumed a condition of greater tonus; toxic effects were also noted on normal hearts, but by no means as pronounced as in sensitized hearts. In 1911 Auer showed independently that the heart of an intact anaphylactic rabbit of sufficiently high sensitization rapidly fails to do its work, and that the animal succumbs for this reason. The functional basis for this heart failure was shown to be a complete or almost complete loss of direct irritability of the heart ventricles. On macroscopical examination of the heart muscle, the right ventricle shows a toughening of the muscle bands on its endocardial surface when scraped by the finger nail. The left ventricle does not show this toughening, except now and then on its papillary muscles. The same observer also demonstrated that these cardiac changes were obtained after section of the vagi, and after destruction of the central nervous system, thus proving that the fatal cardiac reaction was not due to central, nervous influences, but was of peripheral origin. He also noted the absence of any marked disturbance of the lungs in anaphylactic rabbits.

In dogs, Biedl and Kraus, and later Arthus, proved that the main symptom of anaphylaxis was a rapid, profound and long-lasting drop in blood pressure. Experimental evidence led Biedl and Kraus to the view that this fall was due to a paralysis of the vasomotor endings in the splanchnic area. Associated with this drop in blood pressure the same observers noted a diminution in the number of leucocytes, and an extreme loss of coagulability of the blood. Blood drawn from a dog during the anaphylactic state remained fluid for many hours, sometimes days. The respiratory function showed no noteworthy alterations. The dogs recovered as a rule.

The functional alterations produced by anaphylaxis in guinea-pigs is entirely different from those observed in rabbits and dogs. In the guinea-pig, Auer and Lewis showed that the functional interference occurs in the lungs. Within a few seconds after the intravenous injection of an adequate "second" dose the animal shows greater and greater difficulty in getting air into and out of its lungs, until finally a stage is reached where no air at all enters on inspiration and this in spite of the fact that the animal makes most violent inspiratory attempts. Three

to five minutes after the injection respiration ceases and the animal dies of asphyxia. The heart keeps on beating for many minutes after all respiration has stopped. Inspection of the lungs shows a remarkable picture; on opening the thorax, the lungs do not collapse as normal lungs do, but remain fully distended and form a cast of the thoracic cavity (see Fig. 1). Their color is pale bluish pink and the lungs are light in weight. The same lung picture was obtained with equal promptness when the vagi were cut or when the central nervous system was destroyed, thus demonstrating that this lung condition was of peripheral origin and independent of the central nervous system for its production. On the basis of experimental evidence which need not be detailed here, Auer and Lewis conclude that this striking lung condition is produced by a tetanic contraction of the muscles in the finer bronchial tubes. On the basis of this, atropin was used prophylactically with good results, 72 per cent. of the treated animals recovered, while 75 per cent. of their untreated mates succumbed (Fig. 1). The blood-pressure curve in these fatal cases does not resemble that seen in dogs, nor does the blood show a strongly increased coagulation time.

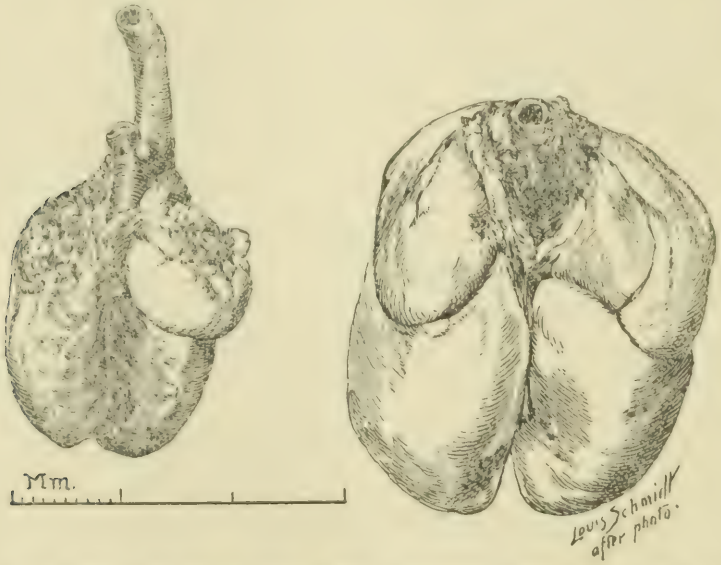


FIG. 1. The large inflated lungs were obtained from a typical fatal case of horse-serum anaphylaxis in a guinea pig. The small collapsed lungs belonged to an anaphylactic guinea pig of the same lot which was saved by the injection of atropin. This animal seemed normal when killed. The picture shows strikingly the characteristic lung picture of anaphylaxis and the remedial effects of atropin.

The right vagus nerve had been resected in each guinea pig thirteen days before the toxic injection.

It will be observed that the important functional disturbances differ in the three species of animals which have been considered above: in the *dog,* the main noticeable effect is a profound, lasting drop in blood pressure associated with a great increase in the time necessary to cause coagulation; the lungs show no lasting inflation. In the *rabbit,* the heart stops beating and the cardiac muscle exhibits a total or almost

total loss of direct irritability to mechanical and electrical stimuli; the lungs show areas of emphysema, but collapse more or less completely when the thorax is opened; the blood shows delayed coagulability, but by no means as great as that observed in a dog. The fall in blood pressure is probably secondary to the failure of the heart. In the *guinea-pig*, the lungs are the chief organs affected and their function is abolished by a stenosis of the finer air passages preventing in the final stage both the entrance and exit of air, so that death results from asphyxia. The anatomical sign of this condition is furnished by the large inflated lungs which do not collapse on excision from the chest cavity (Fig. 1). The heart keeps on beating after final respiratory stoppage, with no obvious loss of irritability; the blood shows only a slight delay in coagulation and the fall in blood pressure is probably due to the fatal asphyxia. These differences between the three species of animals show clearly the necessity of judging each species by the anaphylactic signs characteristic for it and not by manifestations only found in another species. This important point, that each animal species must be measured by its own yard-stick when examined in anaphylaxis, has not been realized, unfortunately, by some investigators.

Causation of Anaphylaxis.—The remarkable phenomena which characterize anaphylaxis early led investigators to search for the causative agent. Numerous theories, more or less supported by experimental facts, were advanced to explain how, for example, the originally harmless horse serum becomes toxic when injected into an animal sensitized by this substance. The pioneer work of Vaughan, Friedemann and Friedberger deals particularly with this aspect of anaphylaxis. A discussion of all the theories here, however, would lead too far and would only befog the reader. It will suffice to state that the basic idea of the chief theory is that the sensitized organism has acquired the power to split the alien serum very rapidly into its components when injected for the second time, and that these components then act as a poison. There can be no theoretical objection to this conception; it is a legitimate working hypothesis. But there are weighty objections just as soon as one substance or mixture of substance is produced from proteids in the test tube by chemical or biological processes and considered as the causative agents of anaphylaxis because when injected into normal animals they produce more or less completely the signs and symptoms which are characteristic of true anaphylaxis. The assumption *may* be true, but no rigid proof has so far been advanced that these substances really are produced in the animal body during anaphylaxis. The mere fact that these toxic substances produce a lesion which also occurs in true anaphylaxis, by no means justifies the conclusion that the causative agents were the same in the two processes. Take, for example, the pale, rigid, distended lungs produced in a sensitized guinea-

pig which succumbs acutely to an intravenous injection of horse serum, and which are diagnostic, when properly considered, of true anaphylaxis in this animal. These lungs owe their state to a tetanic contraction of the bronchial muscles, so that the enclosed air is imprisoned in the alveolar sacs and can not escape even when the lungs are completely excised (see Fig. 1). Now, any adequate stimulus which causes an enduring contraction of these muscles while respiration goes on will produce a greater or less approximation to the lung picture of anaphylaxis. Such adequate stimuli are furnished by a large number of substances, of which we may mention muscarine, eserine, pilocarpine, digitaline, veratrine, morphine barium chloride and the salts of many heavy metals (Dixon and Brodie). Nobody, however, would state that the substances cause anaphylaxis, that they are anaphylatoxins, even though they do produce apparently a lesion of anaphylaxis, for it is perfectly obvious to every one that it is inadmissible to conclude from the identity of reaction produced (in the example chosen, the anaphylactic lung) an identity of the causative agents, as this leads to the ridiculous conclusion that eserine, muscarine, etc., are identical. The same reasoning is applicable to the degradation products obtained by chemical or biological means from proteids. It is not surprising that decomposition products of the infinitely complex proteid molecule should yield substances which are toxic to an organism, and which produce anatomical and functional changes similar to those observed in anaphylaxis, but this does not permit the conclusion that the same decomposition products are formed and exert their actions in true anaphylaxis; such reasoning commits the same error which was mentioned before. It must be insisted that an identity in the biological response caused by a variety of substances permits only the conclusion that these substances are *functionally* identical, not that they are chemically or so to say, morphologically identical. This confusion is widespread, and at present dominant; it is especially due to the *per se* valuable and interesting contributions of Friedberger and his colaborers. Friedberger is convinced that the poisonous mixture which he produces by biological methods in vitro is identical with the causative agent or agents in true anaphylaxis, and in most of his recent work the symptom complexes studied were not true anaphylaxis, but the symptoms produced on first injection by his anaphylatoxin.

The question has probably occurred to the reader why this problem was not approached directly, why, for example, the serum of animals in anaphylaxis was not examined for the presence of these degradation products which are said to play such an important rôle. The test can easily be made, for the split products of proteids which have an albumose or pepton character give the biuret reaction. But no investigator, as far as I am aware, has been able to obtain more than a very feeble or

no reaction from the serum of an animal dead from true anaphylaxis, provided that the test was carried out after the total removal of all coagulable proteids, thus leaving the non-coagulable peptones and albumoses in the filtrate. This method, therefore, gives no evidence of any degradation product demonstrable by the biuret reaction (Pfeiffer and Mita).

The question was attacked in still another fashion by Abderhalden and Pincussohn. If the intoxication of anaphylaxis is produced by the rapid production of toxic cleavage products from the injected proteid, it is legitimate to assume that the serum of sensitized animals should possess ferments which rapidly accomplish this degradation of the proteid molecule. The experimental test was successful in demonstrating proteolytic ferments, but these ferments were not specific nor of very active nature; and later work by Gruber renders their relation to anaphylaxis quite doubtful.

Summing up the evidence which we have regarding the identity of proteid cleavage products and the causative agent or agents of true anaphylaxis, it must be said that while the assumption is theoretically tenable, a firm experimental basis for this assumption is yet to be laid. Moreover, investigators who unreservedly identify the disturbances caused by proteid constituents produced in vitro, with true anaphylaxis, are causing confusion in another direction. Not only is a perfectly well defined symptom-complex like anaphylaxis obscured by this extension of its scope, but a number of characteristic signs of anaphylaxis lose their significance. Before this can be discussed profitably, the original meaning of the word anaphylaxis as well as the functional disturbances and anatomical signs which characterize it, must clearly be kept in mind. On account of the importance of this, it may perhaps be permissible to give a short résumé of matter already discussed.

Meaning of the Word Anaphylaxis, and Diagnostic Criteria.—What the word anaphylaxis was coined to indicate has already been stated; it means the symptoms and signs which are produced when an organism is resubjected to the action of a foreign soluble proteid. When horse serum, for example, is employed, the first injection causes no untoward effects; the second injection, however, gives outspoken and pronounced results which did not occur after the first injection, and these effects are only obtained when a proper interval has elapsed between the two administrations of horse serum. In active anaphylaxis there are three well-defined stages—sensitization, incubation and intoxication. In passive anaphylaxis, where a *normal* animal is sensitized by the injection of the serum of a *sensitized* animal, the same three stages are present, but the period of incubation is now shortened to a few hours. If, therefore, reactions are obtained in an animal after the second or so-called toxic injection which were absent when the first one was given,

we are justified in speaking of the response as an undoubted reaction of anaphylaxis. The three conditions necessary for the employment of this word are fulfilled, and we are dealing with the same phenomena or group of phenomena which the older observers noted and which they called hypersensitiveness, Theobald Smith's phenomenon or anaphylaxis. If these considerations are followed a field of investigation with sharply defined borders is opened up, and every observer is enabled to judge whether or not his particular patch lies within this territory.

These criteria yield a sharply circumscribed mass of phenomena which are undoubtedly caused by the same general process, and which may now be further analyzed without any doubt, whether or not they are of anaphylactic origin. The more obvious signs and symptoms have already been established in dog, guinea-pig and rabbit, which are the animals usually employed in laboratory investigation. But it must be continually borne in mind that the characteristic anaphylactic responses of these three species are characteristic only when they are obtained after the second injection of a soluble proteid; the profound drop in blood pressure in the dog, the large immobilized pale lungs in the guinea-pig and the loss of irritability and contractility of the heart muscle in the rabbit, do not occur when a harmless soluble proteid like horse serum is injected for the first time; they only appear when the injection is repeated after the period of incubation, and this peculiarity characterizes them as anaphylactic and differentiates them at the same time from similar reactions which occur on first injection of a large number of substances.

These considerations render clear, perhaps, why it is not justified at present to admit that those cleavage products of proteids which cause a similar disturbance on first injection really produce true anaphylaxis, for as soon as this assumption is granted the three characteristic conditions of anaphylaxis which give this symptom complex an independent existence by delimiting it from similar complexes, is obliterated. Moreover, the clean and outspoken functional responses found in the three animal species in anaphylaxis lose their diagnostic character and independence, and fall back into the ruck, indistinguishable from a mass of similar reactions. This is surely a heavy price to pay for an extension of the meaning of anaphylaxis, especially as this extension is not necessary. Even when true anaphylatoxins are isolated, no such broadening of the term will be necessary, for only those substances can be considered true anaphylatoxins which are isolated biologically from the tissues and circulatory juices of a case of true anaphylaxis; and these substances must practically not be present in normal animals, but when injected into these normal animals the anaphylactic symptoms and signs characteristic for the species employed must be obtained. Such substances may be the product of proteid cleavage, but they will bear

the name of anaphylatoxins legitimately, for there is no cloud regarding their origin. Such substances, with such a pedigree, have not been isolated so far. With these properly identified anaphylatoxins no confusion will be produced, for they will cause a true anaphylaxis, and the usual conception of this process will not be obscured, but, on the contrary, clarified.

This question of the causal relationship between proteid cleavage products obtained in vitro, and the symptoms and signs of true anaphylaxis has been discussed at some length because this view-point of the problem enjoys great favor; because it was necessary to point out that this view as formulated at present leads to confusion, to the useless sacrifice of a well-defined symptom complex and its characteristic anatomical and functional signs, and finally because this view is not the necessary and inevitable consequence of the experimental data at hand.

Other Manifestations of Anaphylaxis. Serum Disease.—The description given so far has dealt exclusively with the experimental analysis of the more important anaphylactic phenomena in lower animals. But similar phenomena occur in man and these have been extensively studied. Opportunity for this study was afforded shortly after therapeutic sera were generally employed to combat disease, especially diphtheria.[2] To v. Pirquet, associated in his earlier work with Schick, we owe the most thorough study of some of the reactions which the human organism may show when injected with various kinds of therapeutic sera. These reactions v. Pirquet and Schick called serum-disease. The development and course of this serum disease is as follows. The *first* injection of a therapeutic serum, usually obtained from an immunized horse, is tolerated by most individuals without any reaction. In those that do react, the symptoms do not develop at once, but after the lapse of eight to ten days. The chief disturbances which now occur are fever, skin eruptions, swelling of the lymph glands near the site of injection, pains in the joints and muscles and edema of the face and dependent portions of the body. In spite of this apparently formidable list of ailments the general condition of the patient is excellent, as a rule, and there is but slight danger.

When the same patient is reinjected after an interval of more than ten days, the picture is somewhat different. The period of incubation is practically absent or at least considerably shortened, and the symp-

[2] It must be stated at once that these undesirable accessory reactions which sera sometimes show in the human being form no contraindication to their employment in proper cases. In severe diphtheria, for example, the antidiphtheritic serum is the only remedy which can save the patient from death; to hesitate in its employment because it may produce more or less severe symptoms itself, would be criminal, as this action might be equivalent to throwing away the only chance of recovery the patient has.

toms either develop after a few hours, the "immediate reaction," or after a few days, the "accelerated reaction." As the time interval between the injections increases the "immediate reaction" no longer appears, but the "accelerated reaction" still occurs and has even been noted when the second injection followed seven years after the first. The symptoms which characterized the "accelerated" reaction are similar to those already described, with this difference that they occur suddenly, and disappear swiftly within approximately a day. The "immediate" reaction is somewhat different and characterized by a local edema at the site of injection which slowly increases and reaches its maximum within about twenty-four hours, and disappears within two to five days. Associated with this local reaction there is high fever, and the skin shows crops of transitory eruptions of varying character. In a small proportion of all cases the immediate reaction shows a grave picture, there is nausea and vomiting, and at times even collapse.

The similarity between serum disease and anaphylaxis was early noted by v. Pirquet and Schick. The specific local edema, for example, is exactly analogous to Arthus's phenomenon in rabbits; the non-fatal collapse cases also are similar to the results which Arthus obtained in rabbits where he noted a strong drop in blood pressure.

There is another class of severe reactions, fortunately rare, which occur suddenly when a patient is injected for the *first* time with serum. The symptoms bear a striking analogy to those observable in lethal anaphylaxis in guinea-pigs and rabbits. Some individuals show a marked respiratory distress of an asthmatic type with cyanosis, similar to guinea-pigs, and others again show symptoms where the respiratory involvement is not so pronounced, but where cardiac weakness predominates. These cases often end in death. Examination of the history of such individuals often shows that they were subject to asthma, or possessed a peculiar idiosyncrasy to the odor of horses which brought on the symptoms of hayfever and asthma. Cases of this kind are probably examples of anaphylaxis in spite of the apparent absence of any sensitizing injection, for this state of sensitization could easily be attributed to inheritance or to a gradual sensitization via the lungs or the stomach. It is well-known, for example, that a sensitized guinea-pig will transmit this property to her offspring, and we may assume that this also plays some rôle in human cases; moreover Rosenau and Anderson have shown that sensitization may be accomplished in guinea-pigs by feeding raw horsemeat, and more recently Rosenau and Amoss reported that they were able to sensitize guinea-pigs for human serum by injecting the infinitesimal amount of organic material found in the expired breath of human beings. These experimental facts render it quite probable that all these cases where the first injection of horse serum produced alarming symptoms or even death, were sensitized in

one of the ways mentioned to this proteid. It is interesting that those individuals which recover are in a state of anti-anaphylaxis for some time afterwards, that is, they are temporarily free from attacks of asthma or hayfever, from which they suffered before.

Local Reactions of Anaphylaxis.—In 1890 Robert Koch announced that the injection of tuberculin produced a local and general reaction in tuberculous guinea-pigs, which, he said, led to an arrest of the tuberculous process and even to health. Everbody probably remembers the sensation which this statement caused. Unhappily, however, the results obtained later in clinical tests did not fulfil expectations. Nevertheless, one important fact remained: the important diagnostic value of the febrile reaction which follows the injection of tuberculin in the tuberculous individual. This reaction occurs only in subjects which are tuberculous, in other words in those who are sensitized by the proteids of the tubercle bacillus; the reaction is thus one of anaphylaxis.

The original method of injecting tuberculin was not devoid of danger, nor was it at best very agreeable to the individual with a positive reaction. In 1907 v. Pirquet described a cutaneous reaction in tuberculosis which gives accurate results and is devoid of any danger or marked discomfort to the patient. v. Pirquet noted that a very small quantity of tuberculin applied to a local scarification of the skin produced within forty-eight hours a well-marked inflammatory reaction in tuberculous subjects, which did not appear in normal individuals. This inflammatory reaction in an individual shows that he must have been sensitized by the tubercle bacillus, in other words that a tuberculous process is in existence somewhere in the organism. The value of this fact is obvious, for it gives a warning, which if properly heeded may prevent invalidism and death.

A reaction similar to that just described is the ophthalmo-reaction of Wolff-Eisner and of Calmette. Instead of letting absorption take place from the skin these investigators instilled the tuberculin in the conjunctival sac of the eye. In tuberculous subjects a quite violent reaction follows; because of this violent response the ophthalmo-reaction has fallen into disrepute.

Phenomenon of Arthus.—The appearance of a local, massive edema and even necrosis at the site of an injection of serum in a sensitized rabbit was first noted by Arthus in 1903 and has been described before in these pages. In was the first example of a definitely recognized, experimental, local anaphylaxis.

Hayfever.—The most annoying and widespread manifestation of local anaphylaxis is hayfever. Many thousands of people suffer from it in the United States alone. In this country we have two disease periods, the so-called "spring or June cold" prevailing in June and July, and the "autumn catarrh" which begins at the end of July and lasts to October. The majority of hayfever patients suffer during the

latter period, and it is quite exceptional that one individual is afflicted during both periods.

The first attack usually begins near the age of puberty and then recurs every year with such regularity that patients are able to forecast quite accurately the date of their coming illness. The duration of the sickness is approximately six weeks.

The symptoms are those of a catarrhal condition of the mucous membrane of the eyes, nose, pharynx and often of the trachea and bronchi. After a preliminary period of one to two weeks, during which there is a moderate irritation of the eyes and nose, associated with a slight discharge, the disease may reach its maximum within a few days or even hours. At this time the eyes are reddened and swollen, and tear secretion is abundant; the nasal mucous membranes are swollen and injected, causing a copious, watery discharge. In addition there are violent, explosive fits of sneezing together with an intolerable itching, and later a soreness of the nose and the eyes. Subsequently nasal breathing becomes impossible and mouth breathing is necessary. Then irritation symptoms of the larynx, trachea and bronchi develop as shown by attacks of coughing. The lung symptoms vary between a mild bronchitis and a severe asthma.

All these symptoms which make life a burden to the hayfever sufferer are caused by the inhalation of certain pollens. As early as 1831 Elliotson advanced this opinion, and later experimental researches by Blackley and especially by Dunbar placed the theory on a safe footing. Dunbar proved that the albumen fraction of certain pollens was the toxic agent by injecting or instilling this substance in predisposed individuals; under proper conditions the typical symptoms were always obtained. The reaction was specific; thus autumn catarrh patients responded typically to the proteids obtained from ragweed and golden-rod, but showed no effect when the pollen proteid of graminaceous plants was used.

Hayfever patients therefore have acquired in some fashion or another a hypersentiveness to the albumin constituent of certain pollens. When plants bearing these pollens are in bloom, the pollen is distributed by the wind, and when inhaled by susceptible individuals, the typical consequences follow. The specificity of the sensitization explains why some suffer in the spring, when pollen from the flowering grasses is in the air, while others suffer in autumn when golden-rod and ragweed distribute their pollen.

Dunbar has produced a therapeutic serum by inoculating horses with the chief pollen proteids which come into consideration. This pollantin has yielded good results when used prophylactically.

Drug Idiosyncrasies.—There are numerous individuals who react with more or less severe symptoms to drugs which cause no obvious effects in the large majority of people. Among these drugs morphine,

quinine, antipyrine and the iodides may be mentioned. While the history of these cases shows a marked resemblance to anaphylaxis, there is, for most of them, no very definite experimental basis. Friedberger has recently obtained anaphylactic symptoms in guinea-pigs sensitized with an iodine-proteid compound. It would be of theoretical importance if true anaphylaxis could be experimentally produced with these substances, because they are of a non-proteid nature.

Food Idiosyncrasies.—Probably everybody is familiar with the fact that certain foods, harmless for most people, cause marked trouble in others. Certain individuals, for example, react to the ingestion of strawberries, buckwheat, clams, eggs, etc., as if a poison had been swallowed. These cases also are probably anaphylactic, but here again the experimental test is lacking to clinch the relationship between these phenomena.

Treatment of Anaphylaxis.—The treatment is not yet in a satisfactory state, but there are a number of remedies available for some of the manifestations of this protean complex. If an injection of horse serum produces respiratory symptoms of an asthmatic type in a patient, the only rational treatment is the administration of atropin, for the investigations of Auer and Lewis have shown that this asthma is due to a tetanic contraction of the finer bronchioles which hinders or prevents the entrance and exit of air in the lungs, and atropin causes a relaxation of these muscles. In a study of the prophylactic value of atropin injections in guinea-pigs, Auer was able to save 72 per cent. of his animals, while 75 per cent. of the untreated controls promptly died. These results have been corroborated by a number of observers, especially by Biedl and Kraus. The negative results which Friedberger and Mita obtained are probably due to the inadequate dose of the atropin which they administered.

If the injection of the serum, however, causes symptoms of cardiac failure with slight symptoms of asthma, there is no treatment founded on experiment. The treatment must be symptomatic only; but one class of drugs must be avoided. Auer has shown in highly sensitized rabbits that drugs of the digitalis group should not be used, because they hasten the fatal outcome by aiding the production of the same cardiac lesion which anaphylaxis itself calls forth. Moreover, the same observer has recently described changes in the cardiac muscle produced by members of the digitalis group, especially strophanthin, which are very similar to those produced in cardiac anaphylaxis. It is, therefore, clear why these cardiac stimulants must not be given, even though the weak heart would seem to demand their exhibition.

The most rational treatment is the preventive one. The utmost precaution should be observed whenever it become necessary to inject a therapeutic serum in a patient who has been injected with serum before, who is subject to asthma, hayfever, or who shows an idiosyncrasy to

horses. The best treatment of this kind, in my opinion, is the vaccination procedure of Besredka. In this method a very small quantity of horse serum is given subcutaneously or even rectally and time allowed for its absorption. The amount absorbed at any time will be too small to cause serious symptoms and yet enough to produce anti-anaphylaxis. After anti-anaphylaxis has been established, and this occurs quite rapidly, the full dose may be given subcutaneously with some safety. The only objection to this procedure is that time, an hour or two, is lost. The time could be shortened probably, though at some risk. Besredka has obtained good results with the method in guinea-pigs, and it should receive a full clinical trial.

Friedberger and Mita have recently described another method by means of which they were able to protect guinea-pigs against ten times the fatal dose. This result was obtained by a slow intravenous infusion of serum so that only traces enter the circulation at one time. The time of infusion lasted fifty to sixty minutes in their experiments. It will be observed that the same principle used by Besredka, the production of anti-anaphylaxis, is here also utilized.

The treatment for hayfever has already been mentioned.

Importance of Anaphylaxis.—The phenomena of anaphylaxis which have been briefly discussed in the preceding pages are important because they have given us a deeper insight into certain interesting diseases, the so-called idiosyncrasies or predispositions whose causation was formerly inexplicable. The remarkable fact is now established that an organism may be so altered by the injection of an apparently perfectly harmless proteid, that a subsequent injection of the same proteid acts like a violent poison. Predisposition of an individual to any substance means now that this individual is sensitized to this substance. How this sensitization has been accomplished is still undecided in many instances, but the basic conceptions of anaphylaxis will be a safe guide in solving the problem.

It must be emphatically pointed out that the analysis of anaphylactic phenomena would have been impossible without animal experimentation; the chief advances have been made by the functional investigation of these disturbances in laboratory animals and not by tissue examination after death. Thus the autopsies of those early unfortunate cases where death resulted from the administration of a therapeutic serum, yielded no information whatsoever regarding the cause of exitus. The physicians stood before a riddle, the more terrible because its nature was unknown. Animal experimentation has explained this fatal enigma, partially at least, and the physician no longer stands in helpless ignorance before it. He knows the state now, and some methods to prevent or reduce its dangers have been placed at his disposal which promise a fair success.

No. 23

„Separatabdruck aus dem Zentralblatte für Physiologie, Band XXVI, Nr. 4."

(From the Department of Physiology and Pharmacology of the Rockefeller Institute.)

Intratracheale Insufflation im physiologischen Laboratorium.

Von **S. J. Meltzer,** New York.

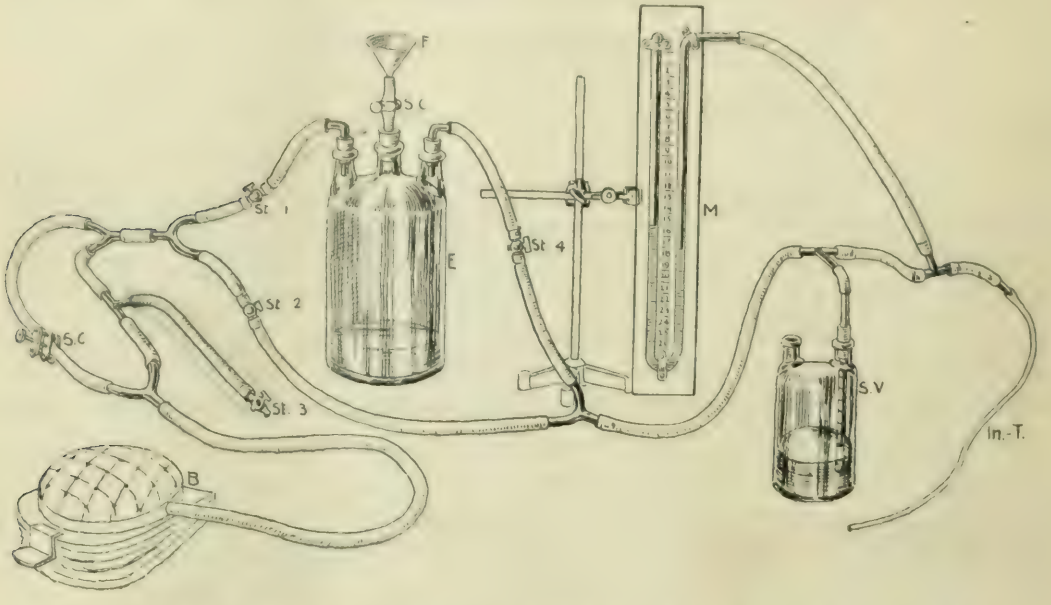
(Der Redaktion zugegangen am 31. März 1912.)

Vor einigen Jahren haben Auer und ich in diesem Zentralblatt[1]) Versuche über „Kontinuierliche Respiration ohne respiratorische Bewegungen" mitgeteilt. In diesen Versuchen wurde ein Rohr durch einen Schlitz in der Trachea von Hunden bis an die Bifurkation vorgeschoben. Bei kontinuierlicher Lufteinblasung durch das Rohr konnten kurarisierte Tiere unbeschränkt lange am Leben erhalten werden. In der betreffenden Mitteilung wurde Gewicht hauptsächlich auf die physiologische Tatsache gelegt, daß nämlich mit der Ausschaltung des toten Raumes bis zur Bifurkation die rhythmischen Atembewegungen ganz entbehrlich werden können; der Vorgang des Gasaustausches geht dann bei der äußern Atmung fast in gleicher Weise vor sich wie bei der innern Atmung. Das diesen Versuchen zugrunde liegende Prinzip wurde jedoch inzwischen zu einer praktischen Methode der künstlichen Atmung ohne Tracheotomie ausgearbeitet[2]). Die Methode wird als intratracheale Insufflation bezeichnet und wird sowohl zur Beseitigung der Schäden des doppelten Pneumothorax als auch einfach für die Administration von Narkose verwendet. Hier in New York allein ist die Methode bis jetzt bei etwa 800 Operationen am Menschen verwendet worden, worauf ich nicht eingehen werde. Hier möchte ich hauptsächlich auf die große Brauchbarkeit der intratrachealen Insufflation für Experimente an Tieren (Hunde und Katzen) hinweisen und will zu diesem Zwecke auf die Ausführung der Methode etwas näher eingehen.

Das Tier wird erst in der gewöhnlichen Weise mit Äther tief narkotisiert und auf dem Operationsbrette befestigt. Das Maul wird weit geöffnet, die Zunge stark herausgezogen und das Lig. glosso-epiglot. wird mit einer gekrümmten Pinzette gefaßt und sanft nach

[1]) Meltzer und Auer, dies Zentralbl., XXIII, 210, 1910.
[2]) Meltzer, Berliner klin. Wochenschr., 1910, Nr. 13.

vorn gezogen, wodurch die hintere, konkave Fläche zugänglich gemacht wird. Auf diese Fläche wird nun das untere Ende eines passenden Katheters gelegt und unter Leitung des Zeigefingers durch den Larynx in die Trachea vorsichtig hinuntergeschoben, bis auf einen Widerstand gestoßen wird; das untere Ende ist dann in der Regel tief im rechten Bronchus. Der Katheter wird darauf etwa 5 bis 6 cm zurückgezogen, wodurch das untere Ende oberhalb der Bifurkation zu liegen kommt. Um die Lage des Katheters zu sichern und denselben zugleich vor den Zähnen des Tieres zu schützen, wird über das äußere Ende des Katheters ein durchbohrter passender Holzstab bis hinter die Schneidezähne des Tieres vorgeschoben, worauf die Schnautze um denselben passend fest gebunden und der Katheter vermittels eines Fadens am Holzstabe befestigt wird. Das äußere Ende des Katheters wird nunmehr mit dem Insufflationsapparate verbunden. — Beistehend gebe ich eine Skizze eines Apparates in der möglichst einfachen Form, um daran einige der notwendigen Bedingungen erläutern zu können.



Erklärung der Bezeichnungen in der Figur.

B. = Blasebalg. St. = Hahn. S. C. = Schraubenklemme.
E. = Ätherflasche. M. = Manometer. S. V. = Sicherheitsventil.
In-T. = Intratracheales Rohr.

Ein Fußblasebalg (B) ist hier die Quelle für einen kontinuierlichen Luftstrom, der durch die erste Gabelung hindurchgeht. An dieser Gabelung ist an der rechten Seite eine Abzweigung mit einem Hahn (St. 3) angebracht, um den Luftstrom einige Male in der Minute für höchstens 2 Sekunden Dauer zu unterbrechen. An der rechten Seite ist eine Schraubenklemme (S. C.) angebracht, um den kontinuierlichen Luftstrom zu verringern und so die Unterbrechung, wenn nötig, verstärken zu können. Auf die erste Gabelung folgt eine zweite für die Regulierung der Narkose. Im linken Aste dieser Gabelung ist eine Äther enthaltende Flasche (E.) eingeschaftet, deren zu- und abführende Schläuche mit Hähnen (St. 1 und St. 4) versehen sind. Der Luftstrom streicht nur durch die obere, mit Ätherdämpfen gesättigte Luftschichte der Flasche. Wenn die Hähne an der linken Seite (St. 1 und St. 4) offen sind und der Hahn am rechten Aste (St. 2) geschlossen ist, dann erhält das Tier „ganz Äther". Wenn die Hähne an der linken Seite geschlossen

sind und der Hahn an der rechten Zeite offen ist, so erhält das Tier „reine Luft". Wenn alle 3 Hähne offen sind, so erhalt das Tier „halb Äther". Durch verschiedene Stellungen der Hähne kann der Grad der Narkose noch weiter abgestuft werden. — Nach Vereinigung der zweiten Gabelung geht der Luftstrom durch einen einfachen Schlauch, der mit dem intratrachealen Rohr (In.-T.) direkt verbunden ist. Dieser Schlauch ist vermittels einer T-Röhre sowohl mit einem Manometer (M.) als auch mit einem Sicherheitsventil (S. V.) verbunden. Das sehr einfache Ventil besteht aus einer Flasche, die Quecksilber enthält, und einer graduierten Röhre, welche mit dem unteren Ende so tief unterhalb des Quecksilberspiegels eingetaucht ist, als man den Druck des Luftstromes innerhalb der Trachea haben will, z. B. 20 mm Quecksilber. Man braucht dann nicht zu befürchten, daß der ungeschulte Gehilfe, dem man sowohl die Öffnung und Schließung des Hahnes (St. 3) als auch das Treten des Blasebalges anvertraut, die Luft mit zu großem Drucke in die Luftröhre treiben würde.

Da bei der Einführung durch den Larynx man das Verhältnis des Durchmessers des Rohres zur Stimmritze doch selten richtig abschätzen kann, so hat die Erfahrung gelehrt, daß man viel sicherer verfährt, wenn man das Rohr eher zu klein als zu groß nimmt. Unter diesen Umständen kann es aber vorkommen, daß, wenn das Tier aus irgend einem Grunde aufhört zu atmen oder, wenn man Kurare gebraucht, die kontinuierliche Luftströmung allein nicht ausreicht, das Leben ungestört lange zu unterhalten. Dieser Übelstand wird aber vollkommen beseitigt, wenn der Luftstrom nur ganz wenige Male per Minute und nur für kurze Zeit unterbrochen wird. Darum wird zur Sicherheit eine solche Unterbrechung konstant bei jeder Insufflation vorgenommen. — Es versteht sich von selbst, daß man anstatt eines Blasebalgs den Luftdruck von einer Luftpumpe und auch die Unterbrechung automatisch besorgen lassen kann, Vorrichtungen, die wohl in den meisten physiologischen Laboratorien vorhanden sind. In unserem Laboratorium steht unser Respirationsapparat in Verbindung mit einem Metallrohr, das der Wand entlang durch zwei Zimmer läuft und mit Verbindungshähnen versehen ist. Wir können so bei mehreren Tieren die Insufflation gleichzeitig vornehmen, was in der Tat sehr häufig geschieht. Ist die Insufflation einmal im Gange und die Narkose richtig eingestellt, so braucht das Tier nur wenig überwacht zu werden. Wir haben in dieser Weise mehrere Tiere 14 Stunden ununterbrochen in der Narkose gehalten, ohne daß viel nach ihnen gesehen wurde und ohne daß sie dabei irgend welchen Schaden davon getragen haben. Ich kann hier auf Einzelheiten und Erklärungen natürlich nicht weiter eingehen. Ich will nur wenige Punkte kurz hervorheben. Das Rohr darf, wie gesagt, nicht zu groß sein. Wir verwenden gewöhnlich einen französischen oder englischen Katheter (oder Magenschlauch) von 5·5 mm Durchmesser (Nr. 16 französisch) für einen Hund von 5 kg Gewicht und 7 mm Durchmesser (Nr. 21 französisch) für einen Hund von 7 bis 8 kg. Stellte es sich heraus, daß das Rohr doch zu klein ausgefallen ist, wodurch dann das Tier nicht tief in der Narkose gehalten werden kann, so genügt es, während einer Minute einige Sekunden hindurch auf das Zungenbein kurz und leicht zu drücken, um den gewünschten Erfolg zu erzielen. — Vor Aspira-

tionen braucht man sich nicht zu fürchten; bei der Insufflation kommt das nie vor. Auch nicht vor dem Äthertod; doch soll man den Äther für kurze Zeit lieber ganz ausschalten, sobald die eigene Atmung des Tieres aufhört. Für die Unterhaltung der Narkose ist überhaupt „halb Äther" genügend. — Wir haben Hunderte von Operationen unter der intratrachealen Insufflation ausgeführt und ich kann nur sagen, daß es eines der hilfreichsten Mittel beim Tierexperiment ist.

Druck von Rudolf M. Rohrer in Brünn.

Sonderabdruck aus d. Biochemischen Zeitschrift 41. Bd., S. 1.

Über die Potentialdifferenzen an der unversehrten und verletzten Oberfläche pflanzlicher und tierischer Organe.

Von

Jacques Loeb und Reinhard Beutner.

(Aus dem Rockefeller Institute, New York.)

(Eingegangen am 18. März 1912.)

I.

Die weitere Verfolgung der Frage nach der physiologischen Wirkung der Salze machte es nötig, auch das Problem der elektrischen Potentialunterschiede zu berücksichtigen, welche bei lebenden Gebilden beobachtet werden. Für die umfangreiche Literatur des Gegenstandes sei der Leser auf die ausführlichen Darstellungen von Hoeber[1]), Max Cremer[2]) und Garten[3]) verwiesen. Auch die spezielleren physikalisch-chemischen Grundlagen dieses Gebietes, insbesondere die Arbeiten von Nernst und Haber und ihren Schülern, müssen wir als bekannt voraussetzen[4]). Hier beschränken wir uns auf die Erwähnung der Arbeiten, welche zu unseren Versuchen in einer unmittelbaren Beziehung stehen.

Die Vermutung, daß die Membranen oder Oberflächenlamellen tierischer Zellen oder Gewebe der Sitz der bioelektrischen Potentialunterschiede seien, wurde zuerst von Wilhelm Ostwald ausgesprochen, der annahm, daß diese Membranen

[1]) Hoeber, Physikalische Chemie der Zelle und der Gewebe. Leipzig 1911.

[2]) Cremer, Nagels Handbuch der Physiologie 4, 868, 1909.

[3]) Garten, Wintersteins Handbuch der vergleichenden Physiologie 1911.

[4]) Den Zusammenhang unserer Versuche mit der Nernstschen Theorie der Reizung wird Beutner in einer besonderen Arbeit besprechen.

einen verschiedenen Grad der Durchlässigkeit für positive und negative Ionen besitzen. Bernstein brachte für diese Annahme eine direkte Stütze. Nach Nernst gilt für die elektromotorischen Kräfte an der Grenze einer wässerigen und wasserunlöslichen Phase die folgende Formel:

$$E = \frac{RT}{nT} \ln \frac{C_1}{C_2},$$

wo T die absolute Temperatur ist.

Bernstein wies nach, daß für den Muskel der Potentialunterschied zwischen einer verletzten und unverletzten Stelle annähernd der Temperatur proportional ist. Die Übereinstimmung war keine ganz vollkommene, was bei der Natur des gewählten Objektes und dem engen Bezirk, innerhalb dessen die Temperatur für den Froschmuskel harmlos ist, nicht zu verwundern braucht. Beim Nerven war die Übereinstimmung weniger befriedigend. Bernstein kam zum Resultat, daß die von ihm beobachteten Erscheinungen ihre Erklärung unter der Annahme finden, daß die Membran durchgängig für die im Muskel enthaltenen Kationen, z. B. K, weniger durchgängig für Anionen (PO_4) ist. Darauf beruhe auch der sogenannte Ruhestrom des Muskels.

Eine Betrachtung der Nernstschen Formel zeigt, daß es noch eine zweite Klasse von Variabeln gibt, welche zur Prüfung der Gültigkeit seines Gesetzes für die bei lebenden Gebilden beobachteten Potentialunterschiede benutzt werden kann, nämlich die Konzentration der Lösungen an der Grenze der Membranen. Der wesentliche Inhalt unserer Arbeit besteht nun darin, daß wir zeigen, daß sich die von der Nernstschen Formel geforderte logarithmische Abhängigkeit der Potentialunterschiede von der Konzentration auch wirklich nachweisen läßt; und zwar in dem Sinne, als ob die Membranen für Kationen „durchlässiger" seien als für Anionen.

Gelegentliche Beobachtungen über den Einfluß der Konzentration finden sich nun schon in früheren Arbeiten, was ja bei einem so vielseitig abgesuchten Gebiet wie das der tierischen Elektrizität ist, nicht zu verwundern ist. Sonderbar ist nur, daß die Beziehung des Konzentrationseinflusses zum Nernstschen Gesetz nicht erforscht oder erkannt wurde. So erwähnt

Biedermann[1]) und nach ihm Oker-Blom[2]), daß ein Muskel bei einseitiger Ableitung mit Wasser auf dieser Seite ein positives Potential annimmt, welchem ein vorübergehendes negatives voraufgeht. (Auch unsere Versuche zeigen, daß mit der Verdünnung der Lösung das positive Potential zunimmt.) Auch Orbeli[3]) beobachtete ein positives Potential bei der Ableitung mit verdünnten Lösungen oder mit Wasser als Ableitung auf einer Seite; er benutzte ganze Frösche als Versuchsobjekt. In allen diesen Versuchen ist jedoch die EMK sehr starken zeitlichen Schwankungen unterworfen und deshalb sind keine exakten Messungen möglich. Eine quantitative Prüfung des Konzentrationseinflusses oder eine Zurückführung auf die Nernstsche Formel findet man daher in diesen Arbeiten nicht. Die Anschauung von dem metallähnlichen Verhalten von Phasengrenzenpotentialen lag den meisten Physiologen fern, da die Hermannsche Alterationstheorie ihre Aufmerksamkeit in eine andere Richtung gelenkt hatte.

Brünings[4]) gibt für den Muskel dasselbe Resultat wie Biedermann und Oker-Blom, nur daß er Zuckerlösungen an Stelle von Wasser zur Ableitung benutzte, wobei dann die mit Zucker in Berührung stehende Stelle des Objektes auch positiv war. Dieser Autor diskutiert die Möglichkeit der Abhängigkeit der EMK von der Verdünnung ausdrücklich, kommt aber zu dem Schluß, daß an physiologischen Membranen eine solche Abhängigkeit nicht besteht. Sein Grund hierfür ist, daß er an künstlichen halbdurchlässigen Membranen eine solche Konzentrationsabhängigkeit nicht nachweisen konnte; bei der Schwierigkeit, vollkommene halbdurchlässige Membranen herzustellen, ist das nicht überraschend.

Der einzige Autor, der eine Beziehung zwischen EMK und Konzentration nachgewiesen hat ist, J. S. Mac Donald[5]). Er maß in der üblichen Anordnung den Längenschnitt-Querschnittstrom von Warmblüternerven. Vor der Messung wurde

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[1]) Biedermann, Sitzungsber. d. Wien. Akad. 81, Abt. 3, 74. 1880.

[2]) Oker-Blom, Arch. f. d. ges. Physiol. 84, 191, 1901.

[3]) Orbeli, Zeitschr. f. Biol. 54, 329, 1910.

[4]) Brünings, Arch. f. d. ges. Physiol. 117, 425, 1907.

[5]) Mac Donald, Proc. Roy. Soc. 67, 310, 1900.

der Nerv einige Minuten lang in eine Lösung bestimmter Konzentration gelegt. Es zeigte sich dann, daß die EMK des Ruhestroms um so größer ist, je kleiner die Konzentration dieser Lösung. Als Elekrolyte wurden NaCl, KCl, NH_4Cl, HCl u. a. verwendet, alle mit dem gleichen Effekt. Er beweist, daß EMK und Konzentration der Spüllösung in logarithmischer Abhängigkeit voneinander sind, welcher er die folgende empirische Form gibt: $E = \text{Const}_1 \log. \frac{\text{Const}_2}{C}$, wo C die Konzentration der Spüllösung ist. Die Nernstsche Formel scheint dem Autor nicht bekannt gewesen zu sein und die empirische Const_1 in Mac Donalds Formel ist eine Funktion einer elektromotorischen Kraft, während sie bei Nernst $= \frac{TR}{F}$ ist. Bei den Bedingungen, unter denen Mac Donalds Versuche ausgeführt wurden, war eine Prüfung der Nernstschen Formel auch wohl kaum durchführbar.

Der Sinn, in welchem sich nach diesen Versuchen die EMK mit der Konzentration ändert, stimmt mit den Angaben der vorhin erwähnten Beobachter überein, wenn man die sicherlich naheliegende Annahme macht, daß der Nerv bei der kurzen Einwirkungsdauer vorwiegend auf der Außenseite mit der Spüllösung behaftet wird. Die Außenseite ist positiv, Anwachsen des Ruhestroms bedeutet also positiver werden der mit der Spüllösung behafteten Membran, und dies tritt bei Verdünnung ein.

II. Methode der Versuche.

Es war aus der Literatur des Gegenstandes ersichtlich, daß Versuche an Muskel und Nerv und vielleicht aller aus dem Innern des Körpers genommenen Organe nicht leicht klare Resultate geben würden. Wir wählten daher Objekte mit kräftiger und dauerhafter Oberflächenmembran, wie die Blätter des Gummibaums, Apfel, Tomaten, und als Beispiele tierischer Objekte die menschliche Hand, und den Frosch. Es wurde darauf geachtet, daß die Objekte völlig unversehrt waren. In der Mehrzahl der Versuche wurde folgendermaßen verfahren: Der Apfel oder die Tomate tauchte mit einem Ende in die Zuleitungsflüssigkeit *a*, welche in einer Schale enthalten war. An

dem andern freien Ende wurde in den Apfel ein kleines Loch gemacht, in welches einige Kubikzentimeter der Zuleitungsflüssigkeit *b* gebracht wurden, so daß wir folgende Anordnung hatten:

Flüssigkeit *a* | Apfel (intakt | verletzt) | Flüssigkeit *b*

Flüssigkeit *b* blieb während einer Versuchsreihe unverändert; die Konzentration der Flüssigkeit *a* (am unverletzten Ende des Apfels) wurde geändert: Wir werden später sehen, warum wir es für nötig hielten die Flüssigkeit bei *b* konstant zu halten und nur die Flüssigkeit an der intakten Außenseite des Objektes zu variieren. Bei Versuchen mit der Tomate verfuhren wir ähnlich; und bei Versuchen mit dem Gummiblatt tauchte der verletzte Stengel in eine Flüssigkeit *b*, die unverändert blieb, während die intakte Spitze des Blattes in die variierende Flüssigkeit *a* tauchte. Die Ableitung geschah mit Kalomelelektroden und die Flüssigkeit in den Elektroden war in verschiedenen Versuchen verschieden, je nach dem Salze, dessen Einfluß auf die EMK untersucht werden sollte. Um eine Diffusion von Salz aus der Elektrodenflüssigkeit in die bisweilen sehr verdünnte Lösung bei *a* zu verhindern, wurde zwischen beide ein Gefäß mit der gleichen verdünnten Lösung geschaltet.

Die Messungen der EMK wurden zum Teil mit der Poggendorfschen Kompensationsmethode (Null-Instrument: Capillar-Elektrometer), zum Teil durch direkte Ablesung mit dem Dolezalekschen Binanten-Elekrometer (Spiegelablesung) bestimmt. Bei dem ersten Verfahren konnte eine Genauigkeit von 1 bis 2 Millivolt erzielt werden, aber wegen des erheblichen inneren Widerstandes der verwendeten Gewebe war die Ausführung häufig schwierig, und die Messung mit dem Elektrometer vorzuziehen.

Das Elekrometer war auf eine hohe Empfindlichkeit eingestellt, so daß 1 mm der Skala etwa 1 Millivolt entsprach, die Fehlergrenze der Messung war etwa 1 Millivolt. Die Aichung konnte bei der großen Empfindlichkeit nicht direkt mit dem Normalelement ausgeführt werden, da die Skala nicht ausgereicht hätte, sondern geschah mit einer kleineren EMK

von 013 Volt, die ihrerseits durch Kompensationsmessung mit dem Normalelement verglichen wurde. Diese Hilfskraft gab einen Ausschlag von ca. 10 bis 12 cm auf der Skala; da der Abstand von Spiegel und Skala 2 m betrug, konnte somit die Tangente dem Winkel proportional gesetzt werden und dieser wieder ohne Bedenken der EMK, da die gesuchten EMKe in der gleichen Größenordnung lagen (ca. 0,1 Volt).

Ein Pol des Instrumentes war geerdet, der andere sorgfältigst isoliert (Aufhängung an mit Paraffin isolierten Haltern). Die Temperatur in unseren Versuchen war stets 18° bis 20°.

Unsere Versuche zerfallen in zwei Gruppen. In der einen Gruppe, zu der die Mehrzahl der Versuche gehört, wurde der Einfluß der Konzentration auf die EMK an der unverletzten Oberfläche gemessen; in der anderen wurde die Messung an der verletzten Oberfläche gemessen. Diese letzteren Versuche werden in einem besonderen Kapitel geschildert.

Änderte man nun in der ersten Gruppe der Versuche die Konzentration an der unverletzten Seite des Apfels oder Blattes, so änderte sich die EMK momentan, d. h. wenigstens so schnell als eine neue Messung ausgeführt werden konnte, und der jeder Konzentration entsprechende Wert blieb annähernd konstant.

Kette:

$$\text{Hg}\,|\,\text{HgCl},\,{}^1/_{10}\text{n-KCl}\;\;\underset{\text{Obere bloß-gelegte Stelle}}{\text{Apfel}}\;\;\underset{\text{Untere unversehrte Stelle}}{{}^1/_{10}\text{ oder }{}^1/_{100}\text{KCl}}\;{}^1/_{10}\text{n-KCl}\,|\,\text{HgCl},\,\text{Hg}$$

$-$ $+$

		Zeit
$^1/_{10}$ n-KCl	0,019 Volt	0′
$^1/_{10}$ „	0,021 „	6′
$^1/_{100}$ „	0,066 „	8′
$^1/_{100}$ „	0,064 „	17′

Differenz der Mittelwerte 0,045 Volt

Wir teilen zunächst die Versuchsresultate bei Änderung der Verletzung an der unverletzten Seite des Apfels mit.

III. Die Gültigkeit des Nernstschen Gesetzes für den Einfluß der Konzentration an der unverletzten Oberfläche.

Wir haben schon erwähnt, daß Bernstein im Anschluß an Ostwald annahm, daß die Oberflächenlamelle des Muskels

für Kationen durchgängig für Anionen undurchgängig ist. Wir wollen zuerst zeigen, daß wenn wir die Konzentration der Flüssigkeit an der Außenseite einer Membran vermindern, diese Seite positiver wird; und zweitens, daß dieselbe, sagen wir etwa 5fache, Verdünnung auch jedesmal dieselbe Erhöhung der elektromotorischen Kraft bedingt, gleichviel ob wir die Konzentration der Zuleitungsflüssigkeit von $^{m}/_{50}$ auf $^{m}/_{250}$ oder von $^{m}/_{250}$ auf $^{m}/_{1250}$ erniedrigen. Bei zu hohen Konzentrationen der Zuleitungsflüssigkeit treten Änderungen der Membran ein, was sich dadurch zeigen läßt, daß nunmehr eine Wiederholung der Versuche nicht mehr die alten Werte für die Potentialunterschiede gibt. Benutzen wir als Zuleitungsflüssigkeit zu niedrige Konzentrationen, z. B. $^{m}/_{6250}$ oder darunter, so kann die Diffusion der Flüssigkeit aus der Kalomelelektrode störend wirken.

Wenn wir den Einfluß berechnen, den eine 5fache Verdünnung der Zuleitungsflüssigkeit a auf der unverletzten Seite des Blattes oder Apfels hat, so ergibt sich derselbe aus der folgenden Formel (unter Voraussetzung, daß die Oberflächenlamelle nur für Kationen durchgängig ist) $\frac{RTna}{Fw}$ ln 5, wobei n die Überführungszahl des Anion, a die Anzahl der Ionen des Moleküls, w die Wertigkeit des Kations bedeutet. Bei dieser Berechnung ist die Änderung des Diffusionspotentiales an der Grenze der Elektrodenflüssigkeit und der Lösung variabler Konzentration mit berücksichtigt. Es ist hierfür erforderlich, daß Elektrodenlösung und Lösung variabler Konzentration dasselbe Salz enthalten; diese Bedingung ist bei den folgenden Versuchen erfüllt. Im Versuch mit KCl enthielten die Elektroden $^{n}/_{10}$-KCl, im Versuch mit NaCl $^{n}/_{10}$-NaCl, im Versuch mit $CaCl_2$ $^{m}/_{10}$-$CaCl_2$, usw.

Wir geben nun eine Reihe von beobachteten Werten und stellen die berechneten daneben. Wir wollen noch einmal bemerken, daß die Konzentration der Zuleitungsflüssigkeit stets auf der intakten Seite des Apfels oder der Tomate oder an der intakten Spitze des Blattes geändert wurde, während die Zuleitungsflüssigkeit an der verletzten Stelle unverändert blieb.

Untersuchungen am Blatt der Gummipflanze (Ficus elastica).

				Differenz beobachtet	Differenz berechnet
$^1/_{10}$	n-KCl	0,013 Volt	>	0,023 Volt	0,040 Volt
$^1/_{50}$	,,	0,036 ,,	>	0,030 ,,	
$^1/_{250}$	,,	0,066 ,,	>	0,033 ,,	
$^1/_{1250}$	,,	0,099 ,,	>	0,031 ,,	
$^1/_{250}$	,,	0,068 ,,	>	0,032 ,,	
$^1/_{50}$	,,	0,036 ,,	>	0,024 ,,	
$^1/_{10}$	,,	0,012 ,,			
$^1/_{1250}$	n-KCl	0,102 Volt	>	0,035 Volt	0,040 Volt
$^1/_{250}$	,,	0,067 ,,	>	0,031 ,,	
$^1/_{10}$	,,	0,036 ,,	>	0,021 ,,	
$^1/_{10}$	,,	0,015 ,,			

Mittelwerte der Differenzen.

				Berechnet
$^1/_{10}$	bis $^1/_{50}$	n-KCl	0,023 Volt	0,039 Volt
$^1/_{50}$	,, $^1/_{250}$	,,	0,031 ,,	0,040 ,,
$^1/_{250}$	,, $^1/_{1250}$	,,	0,034 ,,	0,040 ,,

$^1/_{10}$ n-NaCl 0,030 Volt (Anfangswert)

				Differenz beobachtet	Differenz berechnet
$^1/_{1250}$	n-NaCl	0,141 Volt	>	0,041 Volt	0,049 Volt
$^1/_{250}$	,,	0,100 ,,	>	0,042 ,,	
$^1/_{50}$	,,	0,058 ,,	>	0,029 ,,	
$^1/_{10}$	,,	0,029 ,,			

				Differenz beobachtet	Differenz berechnet
$^1/_{10}$	n-NaCl	0,043 Volt (Anfangswert)			
$^1/_{1250}$	,,	0,141 ,,	>	0,038 Volt	0,049 Volt
$^1/_{250}$	,,	0,103 ,,	>	0,036 ,,	
$^1/_{50}$	,,	0,067 ,,	>	0,024 ,,	
$^1/_{10}$	,,	0,043 ,,			

Dieser zweite NaCl-Versuch wurde an einem anderen Objekt ausgeführt, und deshalb fielen die Resultate etwas anders aus.

				Differenz beobachtet	Differenz berechnet
$^1/_{10}$	m-$CaCl_2$	0,054 Volt			
				0,012 Volt	0,035 Volt
$^1/_{50}$	,,	0,066 ,,			
				0,020 ,,	
$^1/_{250}$	,,	0,086 ,,			
				0,030 ,,	
$^1/_{1250}$	,,	0,116 ,,			
				0,031 ,,	
$^1/_{250}$	,,	0,085 ,,			
				0,020 ,,	
$^1/_{50}$	,,	0,065 ,,			
				0,012 ,,	
$^1/_{10}$	,,	0,053 ,,			

$^1/_{10}$ m-$MgCl_2$ 0,057 Volt (Anfangswert)

				Differenz beobachtet	Differenz berechnet
$^1/_{1250}$	m-$MgCl_2$	0,126 Volt			
				0,033 Volt	0,037 Volt
$^1/_{250}$	,,	0,093 ,,			
				0,023 ,,	
$^1/_{50}$	,,	0,070 ,,			
				0,014 ,,	
$^1/_{10}$	,,	0,056 ,,			

$^1/_{10}$ m-$BaCl_2$ 0,048 Volt (Anfangswert)

				Differenz beobachtet	Differenz berechnet
$^1/_{1250}$	m-$BaCl_2$	0,113 Volt			
				0,029 Volt	0,034 Volt
$^1/_{250}$	,,	0,084 ,,			
				0,022 ,,	
$^1/_{50}$	,,	0,062 ,,			
				0,011 ,,	
$^1/_{10}$	,,	0,051 ,,			

				Differenz beobachtet	Differenz berechnet
$^1/_{1250}$	m-$BaCl_2$	0,118 Volt			
				0,033 Volt	0,034 Volt
$^1/_{250}$	,,	0,085 ,,			
				0,024 ,,	
$^1/_{50}$	,,	0,061 ,,			
				0,013 ,,	
$^1/_{10}$	,,	0,048 ,,			

$^1/_{10}$ n-LiCl 0,055 Volt (Anfangswert)

				Differenz beobachtet	Differenz berechnet
$^1/_{1250}$	n-LiCl	0,158 Volt			
				0,039 Volt	0,055 Volt
$^1/_{250}$	,,	0,119 ,,			
				0,040 ,,	
$^1/_{50}$	,,	0,079 ,,			
				0,026 ,,	
$^1/_{10}$	,,	0,053 ,,			

Jeder einzelne der hier angegebenen Werte stellt einen Mittelwert dar, da jede Messung nach einigen Minuten wiederholt wurde, um die Konstanz zu prüfen. Die Schwankungen

betrugen bisweilen 1 oder 2 Millivolt, meistens änderte sich die Einstellung überhaupt nicht. Alle hier angegebenen Werte sind außerdem, wie man sieht, reversibel, Anfangswert und Endwert jeder Reihe sind innerhalb der Fehlergrenzen gleich. Die Präzision dieser Messungen übertrifft somit die anderer bioelektrischer Messungen ganz erheblich.

An demselben Objekt wurden ferner Versuche mit $ZnSO_4$ und $AlCl_3$ angestellt, welche jedoch nicht reversible Werte lieferten und deshalb an dieser Stelle nicht aufgeführt sind.

Ferner wurden analoge Versuche an Tomaten durchgeführt. Die Konstanz der einzelnen Werte war hier etwas geringer; Schwankungen bis zu 4 Millivolt kommen vor.

Untersuchungen an Tomaten.

				Differenz beobachtet	Differenz berechnet
$^1/_{10}$	n-KCl	0,001	Volt	0,028 Volt	0,040 Volt
$^1/_{50}$	,,	0,029	,,	0,030 ,,	
$^1/_{250}$	,,	0,059	,,	0,032 ,,	
$^1/_{1250}$	,,	0,081	,,	0,032 ,,	
$^1/_{6250}$	,,	0,103	,,		

				Differenz beobachtet	Differenz berechnet
$^1/_{5000}$	m-$CaCl_2$	0,117	Volt	0,040 Volt	0,033 Volt
$^1/_{1000}$	,,	0,077	,,	0,036 ,,	
$^1/_{200}$	,,	0,041	,,	0,028 ,,	
$^1/_{40}$	,,	0,013	,,	0,013 ,,	
$^1/_{8}$	,,	0,0	,,	0,009 ,,	
$^1/_{40}$	,	0,009	,,	0,030 ,,	
$^1/_{200}$	,,	0,039	,,	0,033 ,,	
$^1/_{1000}$	,,	0,072	,,		
$^1/_{1250}$	m-$MgCl_2$	0,102	Volt	0,027 Volt	0,037 Volt
$^1/_{250}$	,,	0,075	,,	0,024 ,,	
$^1/_{50}$	,,	0,051	,,	0,020 ,,	
$^1/_{10}$	,,	0,031	,,		

Versuche am Apfel fielen ähnlich aus und brauchen deshalb hier nicht mitgeteilt zu werden.

Diese Versuche zeigen erstens, daß mit abnehmender Konzentration der Salzlösung an der intakten Oberflächenlamelle der Objekte die Potentialdifferenz immer positiver wird. Aus

dieser Tatsache müssen wir schließen, daß die Membranpotentiale in bezug auf das Kation reversibel sind, oder nach der physiologischen Terminologie, daß die Membran für Kationen, aber nicht oder weniger für Anionen durchgängig ist. Das stützt sich auf die bekannte Erfahrung, daß, wenn wir in einer Kette wie Hg | HgCl, KCl | $CuSO_4$ | Cu | $CuSO_4$ | KCl, HgCl | Hg die $CuSO_4$-Lösung auf einer Seite verdünnen, diese Seite auch positiver wird.

Zweitens zeigen unsere Versuche, daß in Übereinstimmung mit Nernsts Theorie die Differenzen der Potentialunterschiede zwischen einer $^m/_{50}$ und einer $^m/_{250}$ Lösung einerseits und $^m/_{250}$ und $^m/_{1250}$ andererseits gleich sind. Die Differenzen sind auch nahezu dieselben für das Intervall $^m/_{10}$ und $^m/_{50}$, und die Abweichungen lassen sich wohl aus dem nachweisbaren Einfluß der Salze auf die Membranen erklären.

Die gefundenen Differenzen weichen ein wenig von den berechneten ab, aber so gut wie ausschließlich in demselben Sinne: sie sind nämlich alle etwas zu klein. Da nun die Berechnung unter der Voraussetzung erfolgt ist, daß die Membran für Anionen völlig undurchgängig ist, so ist die von uns gefundene geringe Abweichung von den unter dieser Annahme zu erwartenden Zahlen vielleicht dadurch bedingt, daß in Wirklichkeit die Undurchgängigkeit der Membran für Anionen nicht absolut ist.

Endlich zeigen diese und die weiteren Versuche, daß für dieselben Verdünnungen eines Salzes dieselben Potentialunterschiede erreicht werden, gleichviel in welcher Ordnung die verschiedenen Konzentrationen sich folgen. Eine Ausnahme erleidet dieser Satz nur für zu hohe Konzentrationen, worauf wir noch zurückkommen.

IV. Der Einfluß der Anionen.

Der Schluß, daß es sich um Durchgängigkeit für Kationen und relativ geringe Durchgängigkeit für Anionen handelt, läßt sich auch dadurch prüfen, daß wir Natriumsalze mit verschiedenen Anionen vergleichen. Bei der Wahl von Natriumsalzen sollte, wenn es nur auf das Kation ankäme, die EMK bei gleicher Verdünnung stets um denselben Wert geändert werden, gleichviel was das Anion ist; und ferner sollte die

EMK für Natriumsalze mit Anionen verschiedener Wertigkeit dann dieselbe sein, wenn bei der Wahl eines zweiwertigen Anions die Verdünnung halb so groß ist wie bei der Wahl eines einwertigen Anions. Wir beginnen zunächst mit einer Versuchsreihe, welche zeigt, daß das letztere Resultat zutrifft. NaCl gibt dieselbe Potentialdifferenz wie eine zweimal so stark verdünnte Lösung von Na_2SO_4.

Die Versuche sind am Apfel angestellt.

			Differenz beobachtet	Differenz berechnet
$^1/_{10}$	m-NaCl	0,039 Volt		
			0,051 Volt	0,058 Volt
$^1/_{100}$	,,	0,090 ,,		
			0,049 ,,	
$^1/_{1000}$	,,	0,139 ,,		
$^1/_{2000}$	m-Na_2SO_4	0,141 ,,		
			0,049 ,,	
$^1/_{200}$	,,	0,092 ,,		
			0,042 ,,	
$^1/_{20}$	,,	0,050 ,,		

Wie man sieht, entsprechen die EMKe der Theorie. Die weiteren Versuche sind ebenfalls am Apfel angestellt.

			Differenz beobachtet	Differenz berechnet
$^1/_8$	m-NaCl	0,020 Volt		
			0,044 Volt	0,049 Volt
$^1/_{40}$	,,	0,064 ,,		
			0,043 ,,	
$^1/_{200}$	,,	0,107 ,,		
			0,042 ,,	
$^1/_{1000}$	,,	0,149 ,,		
$^1/_{40}$	,,	0,063 ,,		

			Differenz beobachtet	Differenz berechnet
$^1/_{40}$	m-NaBr	0,058 Volt		
			0,045 Volt	0,049 Volt
$^1/_{200}$	,,	0,103 ,,		
			0,041 ,,	
$^1/_{1000}$	,,	0,144 ,,		
			0,042 ,,	
$^1/_{200}$	,,	0,102 ,,		
			0,042 ,,	
$^1/_{40}$	,,	0,060 ,,		
			0,040 ,,	
$^1/_8$	,,	0,020 ,,		
$^1/_8$	m-NaJ	0,048 Volt		
			0,042 Volt	0,049 Volt
$^1/_{40}$	,,	0,090 ,,		
			0,040 ,,	
$^1/_{200}$	,,	0,130 ,,		
			0,043 ,,	
$^1/_{1000}$	,,	0,173 ,,		
			0,047 ,,	
$^1/_{200}$	,,	0,126 ,,		
			0,041 ,,	
$^1/_{40}$	,,	0,085 ,,		
			0,041 ,,	
$^1/_8$	,,	0,044 ,,		

			Differenz beobachtet	Differenz berechnet
$^1/_{250}$	m-NaCl	0,077 Volt		
$^1/_{50}$	..	0,041 ..	0,036 Volt	0,040 Volt[1])
$^1/_{10}$	..	0,006 ..	0,035 ..	
$^1/_{2500}$	m-$Na_2C_2O_4$	0,124 Volt		
$^1/_{500}$	..	0,084 ..	0,040 Volt	0,040 Volt
$^1/_{100}$	..	0,048 ..	0,036 ,,	
$^1/_{20}$	..	0,017 ..	0,031 ,,	
$^1/_{5}$	..	−0,006 ..	0,023 ..	0,035 ..
$^1/_{500}$	..	0,084 ..		

Diese Werte erwiesen sich während einer 2 bis 5 Minuten währenden Beobachtung innerhalb 1 bis 2 Millivolt unveränderlich. Größer waren die Abweichungen bei Natriumcitrat ($Na_3C_6O_7H$), mit dem ebenfalls einige Versuche angestellt wurden, welche hier folgen. In allen Fällen erwies sich die Konzentrationsveränderlichkeit des Potentials gegen KCl oder NaCl veränderlicher Konzentration vor und nach jeder Versuchsreihe als unverändert. Die Schale war also offenbar durch keines der Salze angegriffen.

			Differenz beobachtet	Differenz berechnet
$^1/_{30}$	m-$Na_3C_6O_7H$	0,014 Volt		
$^1/_{150}$	,,	0,034 ,,	0,020 Volt	0,040 Volt
$^1/_{750}$	,,	0,068 ,,	0,034 ,,	
$^1/_{3750}$	,,	0,102 ,,	0,034 ,,	
$^1/_{750}$	,,	0,067 ,,	0,035 ,,	
$^1/_{150}$	,,	0,032 ,,	0,035 ,,	
$^1/_{30}$	,,	0,008 ,,	0,024 ,,	
$^1/_{6}$	,,	−0,011 ,,	0,019 ,,	

Diese Versuche dürfen als weiterer Beleg für die im vorigen Kapitel gezogenen Schlüsse gelten.

V. Versuche mit Nichtleitern.

Wir müssen nun den Einwand betrachten, ob rein osmotische Wirkungen hier in Betracht kommen. Das können wir durch Versuche mit Nichtelektrolyten wie Rohrzucker prüfen,

---

[1]) Bei diesen drei Versuchsreihen war $^n/_{10}$-KCl-Lösung als Elektrodenflüssigkeit verwendet, das Diffusionspotential ist hier bei der Berechnung vernachlässigt und die EMK-Änderung gleich $\frac{RT}{F}$ ln 5 gesetzt.

für welche die Membran denselben Grad der Undurchgängigkeit besitzt wie für Salze.

Es wurde die EMK der Kette

$$\underset{\text{verletzt}}{\text{Hg} \mid \text{HgCl} \mid {}^{m}/_{10}\text{-KCl} \mid \text{Apfel}} \mid {}^{m}/_{100}\text{-NaCl} \mid \underset{\text{intakt}}{{}^{m}/_{10}\text{-KCl}. \text{HgCl} \mid \text{Hg}} +$$

zu 0,092 Volt bestimmt. Darauf wurde der ${}^{n}/_{100}$-NaCl-Lösung so viel Rohrzucker zugefügt, daß die Gesamtkonzentration etwas über ${}^{m}/_{2}$ war. Nachdem aller Zucker sich gelöst hatte und die Lösung gründlich gemischt war, wurde die EMK wieder bestimmt. Sie betrug 0,093 Volt, war also praktisch unverändert. Diese Versuche wurden mit der Tomate wiederholt, in folgender Anordnung:

$$\underset{\text{verletzt}}{\text{Hg} \mid \text{HgCl}, {}^{m}/_{10}\text{-KCl} \mid \text{Tomate}} \mid \underset{\text{unverletzt}}{{}^{m}/_{800}\text{-KCl}} \mid \underset{\substack{\text{mit Rohrzucker} \\ \text{verschied. Kon-} \\ \text{zentrationen}}}{{}^{m}/_{10}\text{-KCl}. \text{HgCl} \mid \text{Hg}}$$

Konzentration des Rohrzuckers	EMK	
${}^{m}/_{500}$	0,093 Volt	
${}^{m}/_{100}$	0,093 „	
${}^{m}/_{20}$	0,093 „	
${}^{m}/_{4}$	0,075 „	
darauf wieder ${}^{m}/_{500}$	0,074 „	nach 10′ 0,084 Volt

Also nur bei hohen Konzentrationen ist eine geringe irreversible Beeinflussung der EMK vorhanden, die vermutlich durch chemische Einwirkung entstanden ist.

Ein ähnlicher Versuch wurde mit Harnstoff als Nichtelektrolyt am Blatt einer Gummipflanze wiederholt.

Konzentration des Harnstoffs	EMK.
${}^{4}/_{125}$ m	0,090 Volt
${}^{4}/_{25}$ m	0,090 „
${}^{4}/_{5}$ m	0,095 „
4 m	0,097 „

Auch diese geringfügige Änderung der EMK war irreversibel; ähnlich verhielt sich das Glycerin.

Konzentration von Glycerin	EMK
0	0,078 Volt
5 mol.	0,082 „

Durch Änderung der Konzentration von Nichtelektrolyten können wir Schädigung der Membran, aber sonst keine Änderung

der EMKe hervorbringen. Die mit reinen Lösungen von Nichtleitern gefundenen Potentialunterschiede sind identisch mit den in reinem Wasser gefundenen, solange die Konzentration der Nichtleiter nicht so hoch ist, daß die Oberflächenlamelle geschädigt wird.

Versuche mit einigen solcher Nichtleiter, welche als typische Anaesthetica wirken, wie Alkohol und Chloroform, ließen dagegen eine sehr erhebliche Wirkung auf das Potential erkennen. Unsere diesbezüglichen Versuche sollen noch fortgesetzt werden, doch läßt sich aus den bisherigen Beobachtungen zweierlei konstatieren: 1. daß Chloroform oder Alkohol, von einer gewissen Konzentration ab der Lösung *a* zugefügt, das Potential erheblich negativer macht. Diese Erniedrigung des Potentials ist um so stärker, je höher die Konzentration an Alkohol ist. 2. Der Einfluß der Konzentration von Salzen auf das Potential wird durch Chloroform- oder Alkoholzusatz nicht verändert.

VI. Einfluß der Wasserstoffionen.

Die Untersuchung der Wirkung der Konzentrationsunterschiede der H-Ionen interessierte uns besonders, weil die Ansicht wiederholt ausgesprochen war, daß gerade diese Ionen für die bioelektrischen Erscheinungen verantwortlich seien. Loeb hatte schon 1898 darauf hingewiesen, daß die Säurebildung bei der Tätigkeit sowie beim Absterben der Organe die Konzentration der H-Ionen in den Zellen vermehren müsse, und daß hier eine Quelle von EMKen vorliege. Aber dieser Autor dachte an Diffusionsströme, welche der Größenordnung nach für diesen Zweck nicht ausreichen. Im Anschluß an die Arbeit von Haber und Beutner untersuchten Haber und Klemensiewiecz die Phasengrenzkräfte der Kette Säure | Glas | Alkali, welche in bezug auf H-Ionen reversibel ist, und stellten die Hypothese auf, daß auch die Phasengrenze der Muskelfibrillen in bezug auf H- und OH-Ionen reversibel sei. Diese sehr ansprechende Ansicht wurde von den Biologen mit Recht als ein Fortschritt angesehen. Wir erwarteten ebenfalls bestimmt, daß unsere Versuche dieselbe bestätigen würden, das war aber in keiner Weise der Fall. Bei gleichgehaltener Gesamtkonzentration ließ sich kein Unterschied zwischen dem Potential saurer und alkalischer Lösungen nachweisen.

Kette:

$$-\text{Hg} \mid \text{HgCl}, \; {}^{m}/_{10}\text{-KCl} \mid \underset{\text{verletzt}}{\text{Apfel}} \mid {}^{m}/_{20}\text{-NaCl} \begin{cases} \text{neutral,} \\ \text{sauer oder} \\ \text{alkalisch} \end{cases} \mid {}^{m}/_{10}\text{-KCl}, \; \text{HgCl} \mid \text{Hg} +.$$

Wir untersuchten zuerst eine neutrale ${}^{m}/_{20}$-NaCl-Lösung, dann eine ${}^{m}/_{20}$-NaCl-Lösung, der so viel NaHO zugesetzt war, daß die Lösung in bezug auf NaHO ${}^{1}/_{1000}$ normal war, und endlich ${}^{m}/_{20}$-NaCl-Lösung, der so viel HCl zugesetzt war, daß die Lösung in bezug auf HCl ${}^{m}/_{1000}$ normal war.

Wir fanden:

${}^{m}/_{20}$-NaCl neutral	0,051 Volt,	während	5′ konstant
${}^{m}/_{20}$-NaCl, ${}^{1}/_{1000}$ n-NaHO . .	0,052 ,,	,,	4′ ,,
${}^{m}/_{20}$-NaCl, ${}^{1}/_{1000}$ n-HCl . . .	0,047 ,,	,,	5′ ,,

Wäre die Membran in bezug auf H-Ionen reversibel, so hätte eine Potentialdifferenz von nahezu 0,5 Volt zwischen sauren und alkalischen Lösungen auftreten müssen. Wir wiederholten den Versuch mit dem Blatt der Gummipflanze.

${}^{m}/_{20}$-NaCl neutral	0,029 Volt,	konstant	während 3′
${}^{m}/_{20}$-NaCl, ${}^{m}/_{1000}$-HCl	0,028 ,,	,,	,, 4′
${}^{m}/_{20}$-NaCl, ${}^{m}/_{1000}$-NaHO . . .	0,031 ,,	,,	,, 4′
${}^{m}/_{20}$-NaCl neutral	0,030 ,,	,,	,, 3′

Die Weiterführung dieser Versuche führte nun zu einem interessanten Resultat. Bekanntlich besteht ein Antagonismus zwischen Säuren und Salzen in bezug auf Schwellvermögen und sonstige Eigenschaften der Eiweißkörper, eine Tatsache, die am eingehendsten von Pauli[1]) und von Procter[2]) untersucht worden ist. Dieser Antagonismus zeigt sich nun auch in diesen Versuchen. Um die Wirkung einer ${}^{m}/_{1000}$-Säurelösung auf Eiweißkörper zu hemmen, ist eine ziemlich hohe Salzlösung nötig. Wir untersuchten den Einfluß einer ${}^{m}/_{1000}$-HCl-Lösung auf das Potential bei niedrigeren Konzentrationen von NaCl als ${}^{m}/_{20}$.

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[1]) Eine Zusammenfassung der Untersuchungen Paulis findet man bei Handovsky, Koll. Zeitschr. 8, 183 u. 267.

[2]) Procter, Koll. chem. Beihefte 2, 243.

$^{m}/_{100}$-NaCl neutral	0,061 Volt,	konstant während 4′
$^{m}/_{100}$-NaCl, $^{m}/_{1000}$-HCl . . .	0,052 „	nach 2′ 0,048 Volt; sinkt weiter
$^{m}/_{100}$-NaCl, $^{m}/_{1000}$-NaHO . .	0,057 „	konstant während 4′
$^{m}/_{100}$-NaCl neutral	0,059 „	„ „ 3′
$^{m}/_{100}$-NaCl, $^{m}/_{1000}$-HCl . . .	0,034 „	nach 3′ 0,029 Volt;
		nach weiteren 4′ 0,028 „
$^{m}/_{100}$-NaCl neutral	0,043 Volt.	

Hier hat also die Säure so gewirkt, als ob sie die Oberfläche modifiziert und für Anionen durchgängiger[1]) gemacht habe. Das wird noch deutlicher, wenn man noch schwächere Salzlösungen anwendet.

$^{m}/_{500}$-NaCl + $^{m}/_{1000}$-NaCl neutral .	0,076 Volt,	konst. während 3′
$^{m}/_{500}$-NaCl + $^{m}/_{1000}$-HCl sauer . .	0,043 „	
	0,030 „	nach 4′
	0,027 „	nach weiteren 3′; sinkt weiter
$^{m}/_{500}$-NaCl + $^{m}/_{1000}$-NaCl neutral .	0,068 „	konst. während 3′

Hier hat also die Säure die spezifische Anionen- resp. Salzdurchlässigkeit noch mehr erhöht als in den früheren Versuchen. Noch deutlicher wird das, wenn wir reine Säure mit reinem Salz vergleichen.

$^{m}/_{1000}$-NaCl neutral	0,088 Volt	
$^{m}/_{1000}$-HCl	0,060 „	
	0,038 „	nach 6′; sinkt weiter.

Alle diese Versuche sind am Blatte der Gummipflanze gemacht. Wir dürfen uns also wohl vorstellen, daß die Säure die Eiweißkörper in der Oberfläche des Blattes modifiziert und damit die Durchgängigkeit dieser Oberfläche für Salze erhöht. Ist aber genug Salz zugegen, so hemmt das diese Modifikation der Eiweißkörper durch Säure und in dem Falle benimmt sich die Säure wie jeder andere indifferente Elektrolyt.

Es stimmt mit dem Gesagten überein, daß niedrige Konzentrationen von Alkali viel schwächer wirken als Säure. Der Sinn der Wirkung ist anscheinend entgegengesetzt wie der von Säure.

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[1]) Da Durchlässigkeit für Kationen schon besteht, bedeutet größere Anionendurchlässigkeit hier dasselbe wie größere Salzdurchlässigkeit.

$^{m}/_{250}$-NaCl	0,055 Volt
$^{m}/_{250}$-NaHO	0,066 ,,
$^{m}/_{250}$-NaCl	0,055 ,,
$^{m}/_{250}$-NaHO	0,062 ,,
$^{m}/_{50}$-NaHO	0,027 Volt
$^{m}/_{50}$-NaCl	0,022 ,,

Alle diese Versuche weisen darauf hin, daß die von uns benutzten Membranen in bezug auf H-Ionen nicht reversibel sind. Wir können im Zusammenhang mit den Beobachtungen über die Wirkung der Säure auf eine Erfahrung zurückkommen, welche wir schon an einer früheren Stelle dieser Arbeit mitgeteilt haben; nämlich daß auch Neutralsalze, wenn ihre Konzentration zu hoch ist, die Membran verändern. Das zeigt sich erstens darin, daß nach der Behandlung der Oberfläche mit einer konzentrierten Neutralsalzlösung die verdünnten Lösungen nicht mehr die alten EMKe geben und zweitens darin, daß bei hohen Salzkonzentrationen die EMKe zu klein ausfallen. Das weist also auf eine erhöhte Salzdurchlässigkeit hin. Hohe Konzentrationen von Neutralsalzen modifizieren also die Oberfläche ebenfalls, nur ist eine viel höhere Konzentration der Neutralsalze erforderlich als im Falle von HCl.

Der Umstand, daß die EMK einer $^{m}/_{20}$-NaCl-Lösung nicht geändert werden, wenn wir sie nacheinander neutral, sauer $^{m}/_{1000}$-HCl und alkalisch $^{m}/_{1000}$-NaHO machen, schließt wohl den Gedanken aus, daß Adsorptionserscheinungen im Spiele sind.

Eine spezifische Wirkung der H· resp. OH′ im Sinne der Nernstschen Gleichung besteht nach dem Erwähnten zwar nicht, bei Abwesenheit aller anderen Elektrolyte wirkt indes Konzentrationsänderung von HCl ebenso wie die von NaCl oder KCl. Die HCl-Konzentration darf hierbei jedoch nicht über $^{m}/_{1000}$ gesteigert werden, da höhere Konzentration die Oberfläche zu stark angreift und die Reversibilität aufhebt. Es wurde z. B. am Apfel gemessen bei

		Differenz beobachtet	Differenz berechnet
$^{n}/_{10000}$-HCl	0,126 Volt	0,061 Volt	0,058 Volt
$^{n}/_{1000}$,,	0,065 ,,		

NaOH wirkt im gleichen Sinne

$^{n}/_{250}$-NaOH	0,062 Volt	0,035 Volt	0,040 Volt
$^{n}/_{50}$- ,,	0,027 ,,		

VII. Versuche an tierischen Objekten.

Alle bisher besprochenen Versuche waren an pflanzlichen Objekten angestellt worden. Wir stellten noch einige Versuche an tierischen Objekten an. Zunächst verglichen wir die Änderung der EMK bei der Änderung der Konzentration der Zuleitungsflüssigkeit an den Fingerspitzen lebender Personen, dem Nagel und der Kuppe der Finger. Der Daumen einer Hand wurde in eine Flüssigkeit getaucht, die während des Versuches nicht gewechselt wurde, während die Zuleitungsflüssigkeit auf dem Nagel des Zeigefingers derselben Hand variiert wurde.

Änderung der Konzentration an der Oberfläche des Fingernagels.

Konzentration der NaCl-Lösung	EMK		Differenz
$^{10}/_{8}$ mol. NaCl	— 0,023 Volt 5′ konst.		
		>	0,017 Volt
$^{1}/_{8}$ „ „	— 0,006 „ 4′ „		
		>	0,035 „
$^{1}/_{80}$ „ „	0,029 „ 6′ „		
		>	0,029 „
$^{1}/_{800}$ „ „	0,058 „		

Wie im allgemeinen wird die Oberfläche mit abnehmender Konzentration der Salzlösung positiver, und bei nicht zu hoher Konzentration entspricht dem gleichen Verdünnungsgrad auch angenähert die gleiche Potentialdifferenz 0,035 Volt zwischen $^{1}/_{8}$ m- und $^{1}/_{80}$ m-NaCl und 0,029 Volt zwischen $^{1}/_{80}$ und $^{1}/_{800}$ m-NaCl. Wir hatten diesen Versuch hauptsächlich unternommen, um zu entscheiden, ob bei Pflanzenmembranen die Harzbestandteile die wasserunlösliche Phase bilden. Beim Nagel handelt es sich aber wohl um Keratin, d. h. Eiweißkörper.

Der folgende Versuch ist an den Fingerspitzen mit Ausschluß der Nägel gemacht.

Änderung der Konzentration an der Oberfläche der Fingerspitze ohne Nagel.

Konzentration der NaCl-Lösung	EMK
$^{10}/_{8}$ mol.	— 0,036 bis 0,028 Volt
$^{1}/_{8}$ „	— 0,031 „ 0,025 „
$^{1}/_{80}$ „	0.011 „ 0,015 „
$^{1}/_{800}$ „	0,028 „ 0,051 „
$^{1}/_{8000}$ „	0.056 „ 0,071 „

Man sieht, daß die EMKe nicht konstant sind, und daß bei den Konzentrationen über $^1/_{800}$ mol. die Differenzen sehr klein sind. Wir haben es also bei der menschlichen Haut mit einer leicht veränderlichen Membran zu tun, die für die Ermittelung der logarithmischen Abhängigkeit der EMK von der Konzentration schon kein gutes Objekt mehr ist. Noch schlagender wird dieses Verhalten in dem folgenden Versuch an der Froschhaut.

1. Versuch am Frosch.

Kette: $^1/_8$ Ringerlösung | Frosch (verletzt / unverletzt) | Ringerlösung wechselnder Konzentration

Konzentration der Ringerlösung an der nicht gehäuteten Oberfläche	EMK	
$^1/_8$ mol.	0,020 bis 0,031 Volt	
$^1/_{80}$ „	0,031 „ 0,046 „	Rechte Seite d. obig. Kette
$^1/_{800}$ „	0,024 „ 0,022 „	—
$^1/_{8000}$ „	0,022 „ 0,016 „	Rechts +
$^1/_{800}$ „	0,022 „ 0,026 „	
$^1/_{80}$ „	0,049 „ 0,048 „	Rechts —
$^1/_8$ „	0,038 „ 0,043 „	
$^1/_{8000}$ „	0,012 Volt	Rechts +

Die EMKe waren starken zeitlichen Schwankungen unterworfen und die Werte sind auch durchaus nicht reversibel. Es läßt sich indes das qualitative Resultat daraus entnehmen, daß von $^1/_{80}$ bis $^1/_{8000}$ mol. ein Positiverwerden mit wachsender Verdünnung eintritt, etwa von der gleichen Größe wie bei den pflanzlichen Objekten, von $^1/_8$ bis $^1/_{80}$ dagegen tritt ein Negativerwerden (von 0,01 Volt pro Zehnerpotenz) ein, dieses Resultat wurde bei Wiederholung bestätigt.

VIII. Versuche an verletzten Oberflächen.

In allen bisherigen Versuchen hatten wir uns nur mit dem Einfluß der Änderung der Konzentration auf die Potentialunterschiede an der unversehrten Oberfläche beschäftigt. Wir wollen nun kurz zeigen, daß der Einfluß der Konzentration sich auch an der verletzten Oberfläche nachweisen läßt; er ist kleiner als an der unverletzten Oberfläche. Wir wollen das

in einer Versuchsreihe an einem Apfel illustrieren. An einer Seite des Apfels war wie gewöhnlich ein Loch in den Apfel gemacht, das mit $^{m}/_{10}$-KCl gefüllt wurde, in welches eine Kalomelelektrode mit $^{m}/_{10}$-KCl eintauchte. Die andere Seite des Apfels tauchte in KCl-Lösungen, deren Konzentrationen nach Potenzen von 10 variierte. Zuerst wurde der Einfluß der Konzentration auf die äußere Cuticula des Apfels wie in allen bisherigen Versuchen gemessen; dann wurde die Cuticula entfernt, wobei so wenig als möglich von der reinen Masse des Apfels mit weggeschnitten wurde, und dann wurde der Einfluß der Konzentration auf diese Oberfläche gemessen. Wir stellen die Werte für dieselben Konzentrationen nebeneinander.

Unverletzte Oberfläche			Verletzte Oberfläche
	$^{m}/_{10\,000}$-KCl	0′	0,035 Volt
		13′	0,043 ,,
		22′	0,047 ,,
0,119 Volt	$^{m}/_{1000}$-KCl	0′	0,024 ,,
0,122 ,,		10′	00,29 ,,
		16′	0,030 ,,
0,065 ,,	$^{m}/_{100}$-KCl	0′	0,008 ,,
0,070 ,,		11′	0,006 ,,
0,020 ,,	$^{m}/_{10}$-KCl	0′	— 0,008 ,,
0,023 ,,		17′	— 0,014 ,,
0,007 ,,	m-KCl		— 0,020 ,,
0,008 ,,			— 0,020 ,,

Der Unterschied im Verhalten beider Reihen wird am besten erkannt, wenn man die Differenzen der Wirkung zweier aufeinanderfolgenden Konzentrationen vergleicht.

Verletzt	Konzentrationsintervall	Unverletzt
0,017 Volt	$^{m}/_{10\,000}$ bis $^{m}/_{1000}$	
0,024 ,,	$^{m}/_{1000}$,, $^{m}/_{100}$	0,052 Volt
0,020 ,,	$^{m}/_{100}$,, $^{m}/_{10}$	0,047 ,,
0,006 ,,	$^{m}/_{10}$,, $^{m}/_{1}$	0,018 ,,

Die beiden Werte 0,052 und 0,047 Volt stimmen mit den früher von uns gefundenen Werten überein und weichen nicht zu sehr von dem theoretisch berechneten Wert 0,058 Volt ab, die Werte für die verletzte Oberfläche, 0,024 und 0,020 Volt, sind

weniger als halb so groß. Auch an der verletzten Oberfläche wird das Potential positiver mit zunehmender Verdünnung. Die verletzte Haut des Apfels verhält sich also zu der unverletzten, wie etwa die unverletzte menschliche Haut zu der unverletzten Haut des Apfels.

Wir geben noch ein zweites Beispiel, an das wir einige Bemerkungen über die Reversibilität dieser Erscheinungen knüpfen wollen. In diesen Versuchen war die Flüssigkeit *b* ($^{m}/_{10}$-KCl), die während der Beobachtungen nicht variiert wurde, nicht in ein in den Apfel geschnittenes Loch gebracht worden, sondern wir befestigten einen Glasring mittels Paraffin auf der unverletzten Haut des Apfels und füllten den Zylinder mit $^{n}/_{10}$-KCl, in welche die eine Kalomelelektrode mit $^{n}/_{10}$-KCl tauchte.

Zuerst wurde eine Versuchsreihe am unverletzten und dann an demselben geschälten Apfel durchgeführt. Wir stellen die Werte wieder gegenüber.

Unverletzter Apfel	Konzentration	Verletzte Oberfläche
0,175 Volt	$^{m}/_{10\,000}$-NaCl	0,056 Volt
0,146 ,,	$^{m}/_{1000}$- ,,	0,036 ,,
0,086 ,,	$^{m}/_{100}$- ,,	0,000 ,,
0,023 ,,	$^{m}/_{10}$- ,,	—0,022 ,,

Die Differenzen sind wieder erheblich kleiner beim geschälten als beim ungeschälten Apfel, aber sonst ziemlich regelmäßig. Es bestehen aber zwei Unterschiede, die in dieser Tabelle nicht zutage kommen. Während die konstante Einstellung beim unverletzten Apfel sehr rasch eintritt, tritt dieselbe beim verletzten Apfel langsamer ein. Ein zweiter Unterschied besteht in bezug auf die Konzentrationsgrenze, über welcher der Apfel geschädigt wird, d. h. seine Reversibilität teilweise verliert. Wir haben schon erwähnt, daß der unverletzte Apfel oder das Blatt, wenn sie mit Lösungen behandelt werden, deren Konzentration höher ist als $^{m}/_{10}$-KCl, wenn sie nachher mit Lösungen von niedriger Konzentration in Berührung kommen, nicht wieder dieselbe Potentialdifferenz zeigen, die vorher beobachtet worden war. Für den geschälten Apfel scheint schon eine $^{m}/_{100}$-KCl-Lösung die obere Grenze zu sein bei der diese Irreversibilität eintritt. Allmählich aber erholt sich auch die verletzte Schicht des Apfels wieder.

Wir wollen einige Beispiele für das Gesagte geben, zunächst ein Beispiel für die Tatsache, daß der Potentialunterschied an der verletzten Stelle oft erst nach einiger Zeit konstant wird. Es ist beachtenswert, daß das nicht für alle Konzentrationen der Zuleitungsflüssigkeit gilt, sondern nur für sehr verdünnte Lösungen.

Enthäuteter Apfel.

Konzentration	Zeit	Potentialunterschied
$^{m}/_{10\,000}$-NaCl	12:15	0,044 Volt
	12:17	0,050 ,,
	12:20	0,055 ,,
	12:24	0,056 ,,
$^{m}/_{1000}$-NaCl	12:27	0,026 ,,
	12:37	0,033 ,,
	12:46	0,036 ,,
$^{m}/_{100}$-NaCl	12:48	0,000 ,,
	12:52	0,000 ,,
	12:53	0,000 ,,
$^{m}/_{10}$-NaCl	12:55	— 0,018 ,,
	12:57	— 0,019 ,,
	12:59	— 0,021 ,,
	1:01	— 0,022 ,,

Nach dieser Versuchsreihe wurden die Bestimmungen wiederholt, aber in der umgekehrten Reihenfolge.

Konzentration	Zeit	Potentialunterschied
$^{m}/_{100}$-NaCl	1:03	+ 0,016 Volt
	1:06	0,015 ,,
	1:08	0,013 ,,
$^{m}/_{1000}$- ,,	1:10	0,006 ,,
	1:45	0,030 ,,

Vergleicht man den letzten Wert für $^{m}/_{100}$-NaCl mit dem vorher erhaltenen, so besteht eine Differenz von etwa 0,016 Volt. Aber um 1:45 ist die Membran wieder normal geworden und der Wert für $^{m}/_{1000}$-NaCl 0,030 ist fast identisch mit den um 12:46 gefundenen, nämlich 0,036 Volt. Daß diese Annahme richtig ist, zeigte eine neue Bestimmung für $^{m}/_{100}$-NaCl:

Konzentration	Zeit	Potentialunterschied
$^{m}/_{100}$-NaCl	2:10	0,002 Volt
	2:12	0,002 ,,

was ja ungefähr der ursprüngliche Wert war.

Man erhält solche Nachwirkungen auch an der unversehrten Membran des Apfels, aber nur, wenn man, wie gesagt, höhere Konzentrationen von Salzen etwa $^{m}/_{1}$-NaCl oder $^{m}/_{1}$-KCl anwendet.

Es ist sehr wohl möglich, daß diese Nachwirkungen bei Organen wie der Muskel sich störend bemerkbar machen.

Die verschiedene Veränderlichkeit des Potentials an der verletzten und unverletzten Stelle könnte den Gedanken nahe legen, die hier beschriebenen Erscheinungen zu einer Theorie des Ruhestromes zu verwerten. Das ist aber kaum berechtigt, wie folgendes Experiment zeigt. Es wurde die EMK der Anordnung

Kalomelelektrode $^{m}/_{1}$-NaCl Apfel $^{m}/_{1}$-NaCl Kalomelelektrode
— intakt verletzt —

in der angegebenen Richtung zu 0,01 Volt bestimmt. Selbst unter der Annahme, daß die verletzte Fläche gar nicht polarisierbar ist, erscheint dieser Befund nicht mit dem Schluß vereinbar, daß alle vorhandenen Potentiale von derselben Natur wie die von uns untersuchten sind, und daß keine inneren, sich unabhängig summierenden Potentiale vorhanden sind. Denn alle vorhergehenden Experimente lehren, daß die verdünntere Lösung immer positiver ist, folglich müßte auch die Ableitungsflüssigkeit auf der linken Seite obiger Kette verdünnter sein als die Zellflüssigkeit des Apfels, d. h. die Konzentration der Elektrolyte des Apfelsaftes müßte mehr als grammolekular sein, was ganz unmöglich ist. Der Umstand, daß die verletzte Stelle ebenfalls eine gewisse Polarisation zeigt, macht einen solchen Erklärungsversuch noch schwieriger. Man sieht also, daß die von uns gefundenen Tatsachen die Erklärung des Ruhestromes nur mit Hilfe von weiteren Annahmen zulassen, deren Besprechung uns zwecklos erscheint.

Was die physikalisch-chemische Seite betrifft, so ist es bemerkenswert, daß alle bis jetzt bekannten Phasengrenzen nur in bezug auf ein einziges Ion reversibel sind, während die von uns untersuchten Objekte für sehr verschiedene Kationen reversibel sind. Entweder enthalten die Oberflächenlamellen alle diese Metalle spurenweise, oder es tritt ein Umtausch zwischen den in der Oberflächenlamelle enthaltenen Kationen und den in der Lösung befindlichen ein.

Zusammenfassung der Resultate.

1. Es wird gezeigt, daß bei Änderung der Konzentration der Lösung eines Elektrolyten an der unversehrten Oberfläche verschiedener pflanzlicher und tierischer Organe die EMK sich in dem Sinne ändert, daß diese Seite mit abnehmender Konzentration positiver wird.

2. Es wird ferner gezeigt, daß die Abhängigkeit der Potentialdifferenz von der Änderung der Konzentration der Nernstschen Formel entspricht. Die wirklich gefundene Potentialdifferenz ist immer ein wenig kleiner als die nach der Nernstschen Formel unter der Voraussetzung berechnete, daß die Membran in bezug auf Kationen reversibel (durchgängig) ist.

3. Aus beiden vorausgehenden Tatsachen wird geschlossen, daß sich die von uns untersuchten intakten Membranen ähnlich wie Metalle verhalten, nur mit dem Unterschied, daß die Durchgängigkeit für Anionen keine absolute ist.

4. Wählt man die Konzentrationen der Salzlösungen zu hoch, so bewirken dieselben Änderungen in der Membran in dem Sinne, als ob dieselbe für Anionen durchgängiger geworden wäre.

5. Bei passender Wahl der Konzentration der Salzlösung (z. B. $^{m}/_{20}$-NaCl) bleibt die Potentialdifferenz die gleiche, wenn die Lösung neutral oder durch Zusatz von HCl $^{m}/_{1000}$ sauer oder durch Zusatz von NaHO $^{m}/_{1000}$ alkalisch gemacht ist. Das zeigt, daß es sich hier nicht um Ketten handelt, die in bezug auf H-Ionen reversibel sind.

6. Konzentrationsänderungen der Zuleitungsflüssigkeit, welche durch Zusatz von Nichtleitern wie Rohrzucker, Harnstoff und Glycerin hervorgebracht sind, haben keinen Einfluß auf die Potentialdifferenz.

7. Es zeigt sich, daß die hier mitgeteilten Gesetze allgemein für intakte Oberflächen gelten, da schon frühere Beobachter angeben, daß bei Ableitung mit destilliertem Wasser von der unverletzten Oberfläche die letztere positiver wird. Dagegen zeigen verschiedene Membranen erhebliche Unterschiede in bezug auf die Wirkung derselben Konzentrationsänderung auf das Potential. Während dieselbe an der intakten Oberfläche von gewissen pflanzlichen Objekten sich dem Maximum nähert, ist sie schon bei der menschlichen Haut erheblich geringer.

8. Entfernt man die Oberflächenlamelle eines Apfels, so gelten die erwähnten Tatsachen ebenfalls, nur mit dem Unterschied, daß die für eine 5fache Verdünnung gefundenen Differenzen der EMK kleiner sind als die an der intakten Oberfläche gefundenen.

9. Die hier mitgeteilte Tatsache, daß Änderung der Konzentration der Salze von Li, Na, K, Mg, Ca, Ba an der unverletzten Membran in gleicher Weise die EMK ändert, widerspricht der Annahme von Bernstein und Hoeber, daß der Ruhestrom durch Diffusion der Salze nur eines Kations (beim Muskel K) entsteht.

10. Der Umstand, daß die von uns untersuchten unversehrten Oberflächenlamellen nicht nur in bezug auf ein Kation, sondern auf beliebig viele reversibel sind, unterscheidet dieselben von allen bisher untersuchten wasserunlöslichen Stoffen, wie Metallen, und den von Haber und Beutner und Haber und Klemensiewicz untersuchten festen Phasen, die immer nur in bezug auf ein einziges Ion reversibel waren.

„Separatabdruck aus dem Zentralblatte für Physiologie, Band XXVI, Nr. 8."

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Anaphylaxie als eine Ursache von Koordinationsstörungen des Herzschlags beim Kaninchen.

Von **John Auer.**

(Der Redaktion zugegangen am 27. Juni 1912.)

In einer Mitteilung über akut tödliche Anaphylaxie beim Kaninchen beschrieb ich Veränderungen des Herzmuskels, die sich mit bloßem Auge nachweisen ließen[1]). Diese Veränderungen sind kurz wie folgt: Wird ein mit Pferdeserum hochsensibilisiertes Kaninchen mit demselben Serum reinjiziert, so erfolgt der Exitus in wenigen Minuten durch Herzlähmung[2]). Nach sofortiger Obduktion sieht man an dem mit Blut gefüllten Herzen, daß die Kammern nur schwach oder gar nicht zucken, während die Vorkammern gewöhnlich langsam und regelmäßig weiterschlagen. Werden die Kammern mechanisch oder elektrisch gereizt, so erfolgt keine oder nur schwache Zusammenziehung. Wird das Herz herausgeschnitten und aufgeschlitzt, so findet man Kammern und Vorkammern mit flüssigem Blut gefüllt und nirgends auch nur eine Spur von Gerinnseln. Jetzt zeigt sich ein merkwürdiger Unterschied zwischen den zwei Ventrikeln: die linke Kammerwand scheint praktisch normal zu sein, was Konsistenz betrifft, aber die rechte Kammerwand fühlt sich steifer und weniger nachgiebig an als normal; wird die Konsistenz mit dem Fingernagel geprüft, so ergibt sich, daß die endokardiale Fläche der rechten Kammer dem Nagel einen Widerstand entgegensetzt, der in ausgesprochenen Fällen an Bindegewebe erinnert. Diese Veränderung ist speziell in den Muskeltrabekeln nahe der Aurikuloventrikulargrenze ausgeprägt. In der linken Kammer hingegen sinkt der Fingernagel leicht in die Muskelmasse und ein sanfter Druck genügt, um Muskelgewebe abzuschaben; nur die Papillarmuskelansätze zeigen eine Resistenz,

[1]) Auer, Journ. of Exper. Med., 1911, XIV, p. 482, 485.

[2]) Siehe Auer loc. cit. für eine nähere Beschreibung der Symptome und für Kurvenbelege. Eine vorläufige Mitteilung erschien dieses Zentralblatt, 1911, XXIV, S. 957.

die jedoch nicht so ausgesprochen ist wie die der rechten Kammerwand. Die mikroskopische Untersuchung von anaphylaktischen Herzen förderte keine markante Differenzen zwischen den beiden Kammern zutage.

Eine so ausgesprochene Resistenz wird nicht in normalen Kaninchenherzen gefunden, obwohl hie und da die Muskeltrabekeln des rechten Ventrikels Andeutungen davon zeigen.

Dieselbe Veränderung von denselben Abschnitten des Herzens wird auch durch letale Gaben von Digitalis, Strophantin[1]) und anderen Giften erzielt.

Da die oben beschriebene grobanatomische Veränderung anscheinend hauptsächlich in einem Teile des muskulären Reizleitsystems, in den Muskeltrabekeln der rechten Herzkammer und den Papillarmuskeln der linken Kammer, lokalisiert ist, schien es wahrscheinlich, daß das Herz Koordinationsstörungen zeigen müßte. Nähere Untersuchung der Kurven, speziell der intrathorakalen Druckschwankungen während eines anaphylaktischen Anfalles, bewies die Richtigkeit dieser Annahme[2]). Koordinationstörungen wurden auch bei der direkten Inspektion des anaphylaktischen Herzens bemerkt (Spaltung des Brustkorbes und künstliche Atmung).

Solche Rhythmusstörungen sind aber nicht nur in tödlichen Fällen von Anaphylaxie beim Kaninchen zu beobachten. In verschiedenen Versuchen, wo das Tier erst nach Stunden oder gar nicht einging, kamen plötzlich einsetzende, temporäre Rhythmusstörungen zum Vorschein. Als Beleg führe ich zwei Kurvenabschnitte an (Fig. 1 und 2). Die obere Reihe in jeder Kurve verzeichnet die Druckschwankungen in der linken Thoraxhälfte (Meltzersche Kanüle und Tambour[3]); Erhöhung des negativen Drucks = Inspiration = Abstieg des Hebels; die mittlere Reihe schreibt den Blutdruck (Hürthles Federmanometer); die gerade Linie ist die Blutdruckabszisse; die Zeitintervalle betragen vier Sekunden.

Zählt man nun die Zacken und Zäckchen, die an der Respirationskurve von Fig. 1 bemerkbar sind, so findet man sie viermal so zahlreich als die Kammerkontraktionen, die das Federmanometer aufschreibt. So z. B. sieht man in einem 12 Sekunden-Intervall, daß die thorakalen Volumschwankungen etwas über 60 Zacken aufweisen, während der linke Ventrikel in derselben Zeit sich nur 15mal zu-

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[1]) Auer, Proc. of the Americ. Physiol. Soc., 1911. Americ. Journ. of Physiol., 1912, XXIX, S. 16.

[2]) Auer, Journ. of Exper. Med., 1911, XIV, Taf. 44, S. 496. Nach einem tonisch-klonischen Krampfanfall sistierte die Atmung und der Mareytambour verzeichnete nur die Volumschwankungen des Herzens; an diesem Kurventeil bemerkt man 3—4 kleine Zacken, wahrscheinlich Vorkammer, und dann eine größere Zacke, eine Kammerkontraktion, da sie auch das Hürthlesche Manometer (Carotis) aufschrieb; Vorkammer und Kammer schlugen also im 3.—4. : 1 Rhythmus.

[3]) Nach Einführung der Kanüle wurde der negative Druck wieder hergestellt.

sammenzieht, wie aus der Blutdruckkurve ersichtlich ist. Da die Zacken der Volumkurve Kammer- und Vorkammervolumschwankungen verzeichnen, sind also nur 45 Zacken auf Vorhofskontraktionen zurückführbar. Unter der Annahme, daß es sich nur um Koordinationsstörungen zwischen Vorkammern und Kammern handelt[1]), zeigt Fig. 1 drei Vorhofszuckungen für jede einzelne Ventrikelkontraktion.

Fig. 2 repräsentiert einen Teil der Kurve fünf Minuten nach Fig. 1. Die Atmung ist stark verlangsamt und während der Exspirationspause kommen die Volumschwankungen des Herzens besonders schön zum Ausdruck. Die großen Schwankungen der Volumkurve in der Atmungspause sind numerisch so zahlreich wie die Pulsdruckzacken des Federmanometers und können deshalb als Ventrikelpulsationen angesprochen werden. Nun bemerkt man aber bei näherer Untersuchung dieser größeren Volumoszillationen, daß jede zwei kleinere Zacken aufweist; eine entfällt auf das Ende der Kammersystole (Hebelabstieg) und die andere auf das Ende der Kammerdiastole (Hebelaufstieg). Hier kann die Sachlage so gedeutet werden, daß je zwei Vorhofkontraktionen auf jede Kammerzuckung entfallen: also ein 2 : 1. Rhythmus.

Zwei Minuten nach Aufzeichnung von Fig. 2 ließen sich keine Störungen der Herzkoordination auffinden; die Volumkurve zeigte nur 2mal so viele Zacken wie die Blutdruckkurve: für jede ventrikuläre Kontraktion war jetzt nur eine Vorhofkontraktion vorhanden.

Eine Stunde nach Injektion des Pferdeserums schien das Tier in gutem Zustande zu sein: der Blutdruck war 98 mm; Respiration 150; rektale Temperatur 39·1°. Dreißig Minuten später verendete das Tier aber plötzlich infolge von Herzstillstand. Der Autopsiebefund des Herzens war typisch und wie oben beschrieben.

Diese Koordinationstörungen des Herzens beim Kaninchen können nicht durch eine Asphyxie erklärt werden, wie z. B. bei der akut tödlichen Meerschweinchenanaphylaxie[2]), denn der Herzschlag, Blutdruck und die Respiration sind ausgiebig genug, um wenigstens das Zustandekommen einer allgemeinen Asphyxie zu verhindern; natürlich ist es aber möglich, daß die Zirkulation im Herzen selber Einflüsse erleidet und eine lokale Asphyxie erzeugt, die sich dann durch einen Herzblock bemerkbar macht. Es ist jedoch wahrscheinlicher, daß die Rhythmusstörungen in der Anaphylaxie, wenigstens beim Kaninchen, durch jene oben beschriebenen anatomischen Veränderungen im Herzmuskel hervorgerufen werden.

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[1]) Ich bin mir wohl bewußt, daß die oben angeführten Tatsachen auch anders ausgelegt werden können, aber mir ist keine Erklärung bekannt, die nicht auf Koordinationsstörungen fußt, und auf den Beweis dieses Faktums kommt es mir hier hauptsächlich an.

[2]) Auer and Lewis, Journ. of Exper. Med., 1910, XII, S. 171 und Fig. 5 auf Taf. IX.

Anhangsweise sei noch erwähnt, daß eine Störung der Herzkoordination (Herzblock) nach Digitalispräparaten (Cushny, Wenckebach, Mackenzie u. a.) vielleicht durch dieselbe Ursache hervorgebracht wird wie bei der Anaphylaxie, da, wie oben schon

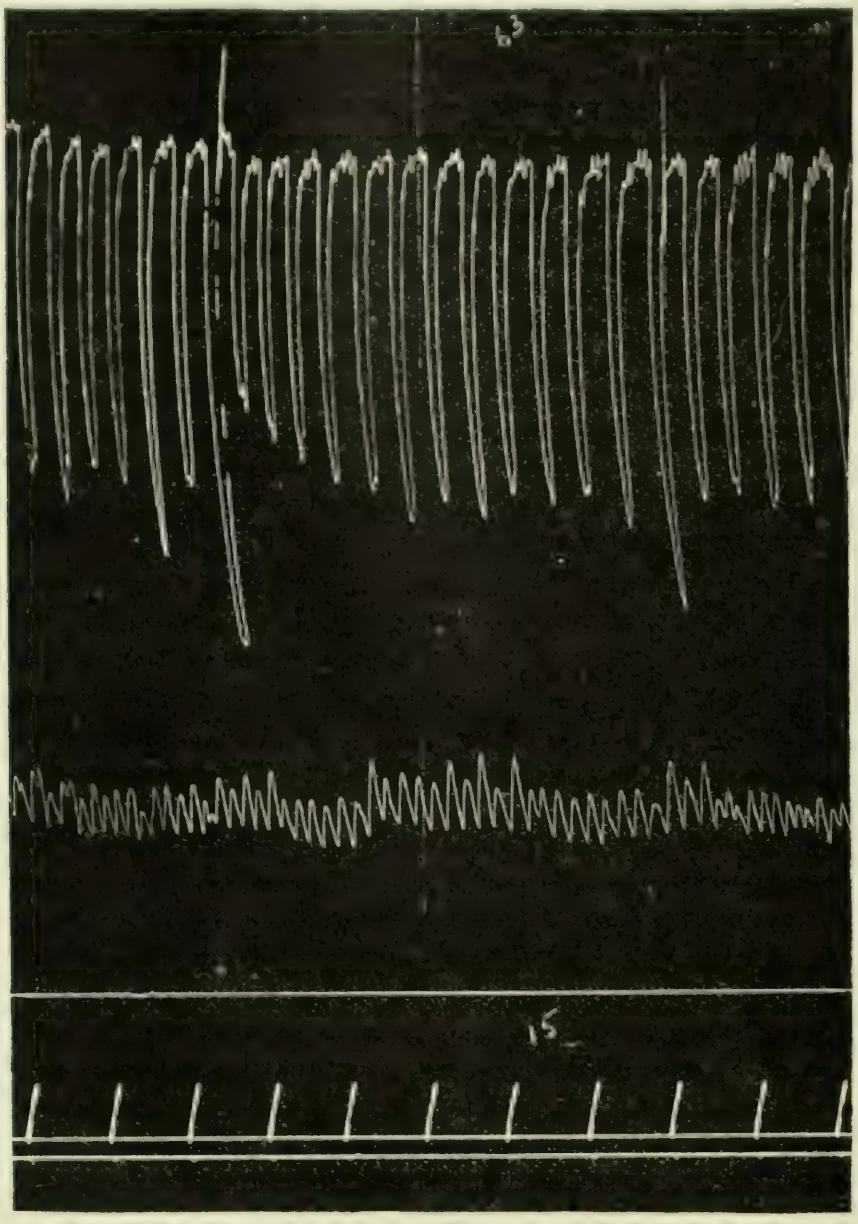

Fig. 1.

erwähnt, diese Drogen beim Kaninchen qualitativ ähnliche Herzveränderungen hervorrufen wie die Anaphylaxie.

Zusammenfassung. Die tödliche und nichttödliche Serumanaphylaxie beim Kaninchen kann temporäre Störungen der Herzkoordination hervorrufen.

In tödlichen Fällen von Anaphylaxie zeigt der Herzmuskel des Kaninchens Verlust von Irritabilität, Kontraktilität und die

Muskeltrakebeln der rechten Kammer sind in ausgesprochenen Fällen zäh und wie gegerbt. Dieselben Veränderungen werden nach letaler Vergiftung mit Digitalispräparaten und anderen Giften erzielt.

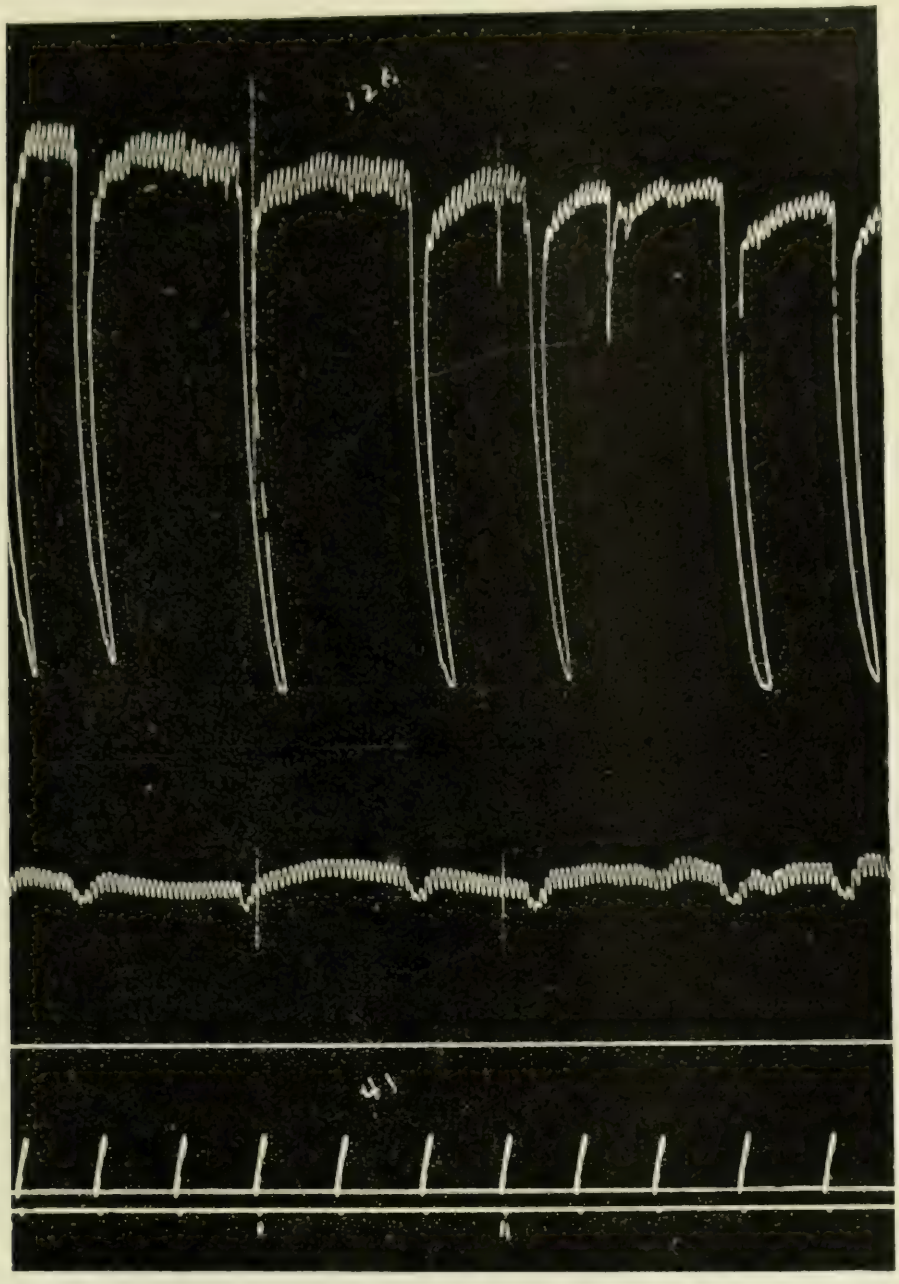

Fig. 2.

Diese anatomischen Veränderungen, in weniger ausgesprochenem Grade, dürften eine Ursache für die Koordinationsstörungen in nichtletalen Fällen von Anaphylaxie und Digitalisvergiftungen beim Kaninchen sein.

NC. 26

Zur Photometrie des Blutfarbstoffes.

Von

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(Aus dem Rockefeller-Institute, New York.)

(Der Redaktion zugegangen am 12. Juni 1912.)

Die Anwendung des Spektrophotometers zur Ermittelung der Konzentration eines farbigen Körpers hat bekanntlich beim Arbeiten mit dem Blutfarbstoff den großen Vorteil, daß man die Bestimmung der Hämoglobinkonzentration ebenso gut mit lackfarbenem Blut als mit reinen Hämoglobinlösungen vornehmen kann, nachdem die erforderliche Konstante der Lichtabsorption des reinen Körpers bekannt ist. Bisher wurde so verfahren, daß aus der Lichtabsorption einer ca. 100fach verdünnten Lösung auf die Konzentration der konzentrierten Lösung geschlossen wurde. Dabei mußte die Gültigkeit des Beerschen Gesetzes für große Konzentrationsintervalle vorausgesetzt werden. Dies würde notwendigerweise eine weitere Annahme in sich schließen, nämlich die, daß bei der Verdünnung des Blutfarbstoffs weder konstitutive Änderung noch Dissoziationsvorgänge, welche die Lichtverteilung im Spektrum ändern würden, auftreten. Nun ist gerade in den letzten Jahren die Gültigkeit des Beerschen Gesetzes einerseits angezweifelt worden, und anderseits glaubt Manchot[1]) annehmen zu müssen, daß im unverdünnten Blut bei normalem Luftdruck beträchtliche Mengen ungesättigten Hämoglobins neben Oxyhämoglobin enthalten seien. Die Feststellung der Grenzen, innerhalb deren das Beersche Gesetz gültig ist, würde von praktischer Bedeutung für die Hämoglobinbestimmung sein. Besonders mußte dabei auf die

[1]) Ann. d. Chem., Bd. 370, S. 280 (1909).

Lichtabsorption konzentrierter Lösungen geachtet werden; denn es wäre wünschenswert, bei künftigen Dissoziationsstudien die spektrophotometrischen Messungen direkt an den dazu verwendeten, meist konzentrierten Lösungen vorzunehmen. Ferner würde man Aufschlüsse über die eventuelle Verschiebung des Verhältnisses Hämoglobin zu Oxyhämoglobin erhalten, die bei der Verdünnung des Blutes unter gleichbleibendem Sauerstoffdruck auftritt.

Ich habe es daher unternommen, die Lichtabsorption des Blutfarbstoffs innerhalb sehr weiter Konzentrationsgrenzen zu untersuchen. Es hat sich ohne weiteres herausgestellt, daß das Beersche Gesetz innerhalb der untersuchten Grenzen streng gültig ist. Dieses Ergebnis besagt, daß Änderungen des Verhältnisses Hämoglobin zu Oxyhämoglobin ausgeschlossen sind. Neuerdings haben Heubner und Rosenberg[1]) mittels einer sinnreichen photographisch-photometrischen Methode die Lichtverteilung im Spektrum des 10-, 60- und 220fach verdünnten Kaninchenblutes untersucht. Sie konnten dabei «ein Argument für die Richtigkeit der Ansicht Manchots nicht gewinnen». In der Arbeit Manchots waren jedoch die Unterschiede in der Sauerstoffbindung am ausgeprägtesten zwischen dem unverdünnten und dem 10fach verdünnten Blut. Um die Ergebnisse der spektrophotometrischen Messungen mit denen der gasanalytischen Methoden zu vergleichen, müßte man noch die Messungen der Lichtabsorption an derselben Konzentrationsreihe wie Manchot vornehmen.

Ich möchte bei dieser Gelegenheit zu der Arbeit von Heubner und Rosenberg bemerken, daß überall dort, wo unsere Beobachtungen zusammenfallen, die erfreulichste Übereinstimmung zu konstatieren ist. Besonders gilt dies für ihre Werte für $\frac{\epsilon \text{ max. Grün}}{\epsilon \text{ min. Gelb}}$. In einer früheren Untersuchung[2]) habe ich eine Erklärung dafür gegeben, warum der Quotient $\frac{\epsilon'}{\epsilon}$ von Hüfner keine absolute Konstante sein konnte. Der Wert des Quotienten nämlich ist bei den bisherigen Versuchen von der Reinheit des Spektrums, von der Breite des herausgeschnittenen Spektralgebietes,

[1]) Biochem. Zeitschrift, Bd. 38, S. 345 (1912).
[2]) Diese Zeitschrift, Bd. 62, S. 198—204 (1909).

sowie auch von wohl unvermeidlichen Fehlern in der Konstruktion des Apparates abhängig. Der Quotient wird erst dann einen konstanten Wert annehmen, wenn die Absorption bei einer einzelnen Wellenlänge mit fehlerfreiem Apparat gemessen wird. Da nun der Quotient $\frac{\epsilon'}{\epsilon}$ das Verhältnis der durchschnittlichen Absorption im Bereich eines Maximums zu der eines Minimums darstellt, so ist es ohne weiteres klar, daß der höchste Zahlenwert des Verhältnisses erreicht wird, wenn genau bei der Wellenlänge des Maximums, sowie genau bei der des Minimums gemessen wird. Deshalb habe ich früher mit dem Apparat von Martens und Grünbaum die Lage des Maximums und des Minimums photometrisch bestimmt und das Verhältnis $\frac{\epsilon \text{ max. Grün}}{\epsilon \text{ min. Gelb}}$ bei möglichst kleinen Kollimator- und Okularspaltbreiten gemessen. Bei Rinderhämoglobin und Menschenblut erhielt ich den Wert 1,67.[1]) Nun haben Heubner und Rosenberg den gleichen Wert für $\frac{\epsilon \text{ max. Grün}}{\epsilon \text{ min. Gelb}}$ auf photographischem Weg erhalten.

Bei der vorliegenden Untersuchung wurden die Messungen mit dem Apparat von König, Martens und Grünbaum an Lösungen von Oxyhämoglobin, sowie auch an lackfarbenem Blut bei Zimmertemperatur gemacht. Als Beleuchtungsquelle diente meist die Cooper-Hewittsche Quecksilberlampe, seltener die Na-Flamme. Kleine Schichtdicken wurden dadurch erreicht, daß man dünne Metallringe (0,018 cm bis 0,117 cm) zwischen die planparallelen Glasplatten brachte und das Ganze in der Fassung des 2 cm langen Absorptionsrohres fest einschraubte. Die Reflexionen wurden mit einer gleich dicken Schicht Wasser zwischen Glasplatten kompensiert.

Es darf nicht verschwiegen werden, daß die Justierung des Apparates besonders wegen der Beleuchtungsvorrichtung ungemeine Sorgfalt erfordert. Bei den kaum zu vermeidenden Fehlern wird, obwohl alle Vorsichtsmaßregeln, wie Kontrollierung des Strahlenganges, Eliminierung von Asymmetrien, Kompensation der Reflexionen usw., getroffen wurden, kein Anspruch auf absolute Zahlen erhoben. Es genügt vielmehr für den Zweck dieser Arbeit, daß die Extinktionskoeffizienten verschieden konzentrierter Lösungen desselben Präparates unter sich vergleichbar sind. Deshalb wurde bei einem gegebenen Blut- oder Hämoglobinpräparat die Reihe von Messungen bei unveränderter Justierung des Apparates, der

[1]) Butterfield, l. c., S. 201.

[2]) Heubner und Rosenberg, l. c., S. 375.

Beleuchtungsvorrichtung und Lichtquelle vorgenommen und ohne Unterbrechung durchgeführt.

Die Bezeichnungsweise ist die übliche.[1]) α_2 = Stellung des Nicols bei gleicher Helligkeit der Felder, absorbierende Lösung rechts, Wasser links; α_1 = Ablesung nach Vertausch der Absorptionsröhre. c = Konzentration (meist relativ ausgedrückt), d = Schichtdicke, ϵ = Extinktionskoeffizient.

Tabelle I.

Oxyhämoglobin vom Rind.
Konzentration der Stammlösung = 9,96 g in 100 ccm Wasser.
Beleuchtung mit Na-Flamme.

Wellenlänge μμ	Konzentration relativ c	Schichtdicke cm	α_2 Grad	α_1 Grad	Extinktionskoeffizient ϵ
589	1	0,049	9,17	65,18	23,0
589	1/40	2,00	9,13	65,60	22,7
589	1/80	2,00	16,71	47,99	22,7

Tabelle II.

Lösung gewaschener Blutkörperchen vom Schaf.
Stammlösung = 1 Volumenteil Blutkörperchenmasse + 1 1/2 Volumenteile Wasser. Beleuchtung: Hg-Lampe.

Wellenlänge μμ	Konzentration relativ c	Schichtdicke cm	α_2 Grad	α_1 Grad	Extinktionskoeffizient ϵ
546	1	0,047	4,82	84,08	43,8
546	1/40	2,00	4,20	84,96	43,8
577 / 579	1	0,047	3,74	85,72	49,2
577 / 579	1/40	2,00	3,30	86,28	48,5

c	$\frac{\epsilon_{577,\ 579}}{\epsilon_{546}}$
1	1,12
1/40	1,11

[1]) Martens u. Grünbaum, Ann. d. Physik, 4. Folge, Bd. 12, S. 984.

Aus diesen Zahlen geht deutlich hervor, daß das Beersche Gesetz innerhalb des untersuchten Konzentrationsgebietes auch bei verschiedenen Wellenlängen streng gültig ist. Es ist ohne weiteres klar, daß der Quotient irgend eines Paares dieser Extinktionskoeffizienten bei den untersuchten Konzentrationen konstant ist; wahrscheinlich ist dies der Fall bei allen zwischenliegenden Konzentrationen. Die untersuchten Konzentrationen entsprechen einem Oxyhämoglobingehalt von ca. $^1/_2$—$^1/_{80}$ bezw. $^1/_{160}$ des nativen Blutes.[1]) Mit Rücksicht auf die Arbeit von Manchot mußte besonders auf die Konzentrationen im Bereiche einer 10fachen Verdünnung des nativen Blutes geachtet werden, eine Konzentration, die bei den oben angeführten Messungen übersprungen wurde. Bei 10facher Verdünnung tritt nämlich nach Manchot ein Maximum der Sauerstoffbindung des Blutes auf. Die Zunahme des gebundenen Sauerstoffs ist bei dem 10fach verdünnten Blut (bei 0° und Atmosphärendruck) ca. 50% des Anfangwertes des unverdünnten Blutes. Bei 36,5° geht die Zunahme auf 20% zurück. Wenn dieses Phänomen tatsächlich von beträchtlichen Mengen ungebundenen Hämoglobins neben Oxyhämoglobin im nativen Blut herrührt, so müßte man die Menge des noch ungebundenen Hämoglobins mit dem Spektrophotometer bestimmen können; denn die Lichtverteilung im Spektrum der beiden Farbstoffe ist hinreichend verschieden, um die Feststellung der prozentischen Zusammensetzung eines Gemisches durch photometrische Messungen bei passend gewählten Wellenlängen zu ermöglichen. Der Quotient $\frac{\epsilon_{577,\ 579}}{\epsilon_{546}}$

[1]) Es wurden bis jetzt keine Messungen bei einer Konzentration, welche mit dem Hämoglobingehalt des unverdünnten Blutes gleich wäre, vorgenommen, obwohl dies keine Schwierigkeiten macht. Es gelingt sehr leicht, auf folgende Weise klare konzentrierte Lösungen, welche mit Dunkelfeldbeleuchtung optisch leer sind, herzustellen. Man versetzt 1 Volumenteil gewaschener Blutkörperchen mit 1 Volumenteil destillierten Wassers, kühlt auf 0° ab und schüttelt mit Äther aus. Nach einiger Zeit setzt sich eine klare wässerige Lösung von Oxyhämoglobin ab. Der Äther kann von der wässerigen Lösung durch einen Strom gewaschener Luft entfernt werden. Nach diesem Verfahren ist es möglich, klare Lösungen zu gewinnen, welche oft 20 g Oxyhämoglobin in 100 ccm enthalten.

ist beispielsweise 0,776 für sauerstofffreies Hämoglobin vom Hunde, während dasselbe Hämoglobin mit Sauerstoff gesättigt einen Quotient $\frac{\epsilon_{577,\ 579}}{\epsilon_{546}}$ von 1,10 aufweist. Der Quotient von Gemischen der beiden Farbstoffe hat bekanntlich dazwischen liegende Werte.

Es seien zunächst die Messungen bei einer ziemlich vollständigen Verdünnungsreihe wiedergegeben.

Tabelle III.

Hundeblut.

Verdünnungsmittel: Wasser. Beleuchtungsquelle: Hg-Lampe.

Verdünnung	Schichtdicke cm	$\frac{\epsilon_{577,\ 579}}{\epsilon_{546}}$
2,0	0,018	1,10
2,5	0,018	1,10
3,75	0,033	1,10
5,0	0,058	1,10
7,5	0,072	1,10
10,0	0,094	1,10
12,5	0,094	1,10
15,0	0,117	1,10
200,0	2,00	1,10

Mit krystallisiertem Oxyhämoglobin aus Hundeblut erhielt ich gleiche Resultate.

Schließlich seien einige Bestimmungen an Kaninchenblut angeführt. Der Wert des Quotienten $\frac{\epsilon_{577,\ 579}}{\epsilon_{546}}$ für sauerstofffreies Hämoglobin war bei diesem Blut 0,780.

Tabelle IV.

Kaninchenblut.

Verdünnungsmittel: Wasser. Beleuchtungsquelle: Hg-Lampe.

Verdünnung	Schichtdicke cm	$\frac{\epsilon_{577,\ 579}}{\epsilon_{546}}$
10,0	0,072	1,12
12,5	0,094	1,12
15,0	0,117	1,12
200,0	2,00	1,13

Es könnten noch andere Bestimmungen an Lösungen von Rinderblut, sowie an Blut oder Oxyhämoglobin des Pferdes angeführt werden. Ich glaube jedoch, daß das Resultat der schon angeführten Bestimmungen zur Genüge eindeutig und hinreichend allgemein gültig ist, um theoretische Einwände gegen die spektrophotometrische Hämoglobinbestimmung zu widerlegen. Die Unveränderlichkeit des Spektrums des Oxyhämoglobins bei der Verdünnung mit Wasser, welches mit Luft bei Atmosphärendruck gesättigt ist, schließt eine Verschiebung des Verhältnisses $\frac{\text{C-Hämoglobin}}{\text{C-Oxyhämoglobin}}$ unter diesen Versuchsbedingungen aus. Dies war wohl nach dem Massenwirkungsgesetz unter Annahme einer einfachen Beziehung, wie 1 Mol. Hämoglobin + n Mol. Sauerstoff = 1 Mol. Oxyhämoglobin, vorauszusagen. Es müßte allerdings noch die weitere Annahme gemacht werden, daß die Löslichkeit des Sauerstoffs durch die Konzentration des Blutfarbstoffs nicht wesentlich geändert wird.

Sonderabdruck aus d. Biochemischen Zeitschrift 43. Bd., S. 181.

Über die Hemmung der Giftwirkung von NaJ, $NaNO_3$, NaCNS und anderen Natriumsalzen.

Von

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(Aus dem Rockefeller Institute, New York.)

(Eingegangen am 6. Juni 1912.)

I.

In früheren Arbeiten hatte ich die Frage aufgeworfen, wie es komme, daß eine Mischung von $NaCl + KCl + CaCl_2$ in dem Verhältnis, in dem diese Salze im Seewasser enthalten sind, am besten für die Erhaltung des Lebens der Zelle ist, und war zu dem Schluß gekommen, daß die Bedeutung dieser Salze darauf beruht, daß dieselben der Oberflächenlamelle der Zelle oder des Tieres den Grad der Dauerhaftigkeit und Undurchgängigkeit verleihen, der für die Erhaltung des Lebens nötig sei[1]). Diese Annahme erklärt den Umstand, daß eine reine NaCl-Lösung, sobald ihre Konzentration eine gewisse Grenze übersteigt, giftig ist, während dieselbe durch Zusätze von $CaCl_2$ und KCl im richtigen Verhältnis harmlos wird. Die reine NaCl-Lösung verändert die Oberflächenlamelle der Zellen, dringt infolgedessen in die Zellen (resp. das Tier) und schädigt dieselben; während der Zusatz von $CaCl_2$ und KCl die Oberflächenlamelle für NaCl relativ undurchgängig macht. Ich konnte ferner zeigen, daß eine reine KCl-Lösung von der Konzentration, in der dieses Salz im Seewasser vorhanden ist, die marinen Fische Fundulus rasch tötet, während der Zusatz von NaCl oder $CaCl_2$, oder von beiden, in bestimmtem Verhältnis, die Tiere am Leben erhält[2]). Die Erklärung war wieder die

[1]) Arch. f. d. ges. Physiol. **107**, 252, 1905; Science **34**, 653, 1911; diese Zeitschr. **36**, 277, 1911.

[2]) Diese Zeitschr. **31**, 450, 1911.

daß eine reine KCl-Lösung leicht in die Tiere diffundiert und die Nerven und Muskeln vergiftet; während der Zusatz von NaCl oder $CaCl_2$, oder von beiden, die Diffusion von KCl in das Tier verhindert. Es waren noch eine Reihe von anderen Beweisen gegeben worden, für die der Leser auf die betreffende Abhandlung verwiesen sei.

Wenn die Annahme richtig ist, daß die drei Salze NaCl + KCl + $CaCl_2$ in dem Verhältnis, in dem sie im Seewasser enthalten sind, die Durchgängigkeit der Fische für Salze zu einem Minimum machen, so sollte man erwarten, daß artfremde Salze und Ionen in einer solchen Mischung weniger rasch in die Fische diffundieren als in irgendeiner anderen Kombination von Salzen. Indirekt läßt sich diese Annahme dadurch prüfen, daß man versucht, ob die Fische in einer Mischung von NaCl + KCl + $CaCl_2$ eine höhere Konzentration eines artfremden oder schädlichen Salzes ertragen können als in irgendeiner anderen Kombination von Salzen. Die Resultate solcher Versuche sollen im folgenden mitgeteilt werden.

Zur Methode der Versuche sei folgendes bemerkt. Fundulus ist in weiten Grenzen unabhängig vom osmotischen Druck der umgebenden Lösung. Die Fische leben beliebig lange in einer $^m/_8$-Lösung von NaCl + KCl + $CaCl_2$ in dem Verhältnis, in dem diese Salze im Seewasser vorhanden sind, oder in einer $^m/_8$-Lösung von NaCl, oder in einer Lösung von 5 ccm $^m/_2$-NaCl in 100 ccm H_2O, oder in einer Lösung von 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O. Es wurden nun Lösungen artfremder Salze, z. B. NaJ oder NaCNS oder $NaNO_3$ und andere hergestellt, und zwar in destilliertem Wasser mit und ohne Zusatz anderer Salze. Es wurde dann ermittelt, in welchen Lösungen die Fische die höchste Konzentration des giftigen Salzes ertragen konnten. Es stellte sich nun heraus, daß in allen Fällen die Fische in einer $^m/_8$-Lösung von NaCl + KCl + $CaCl_2$ (im Verhältnis, in dem diese Salze im Seewasser enthalten sind) eine höhere Konzentration des giftigen Salzes ertragen als in irgendeiner anderen Lösung. Die Lösungen, die auf ihre gifthemmende Wirkung verglichen wurden, waren solche, in denen die Fische ohne den Zusatz der artfremden Salze beliebig lange am Leben bleiben.

Ehe die Fische in die Lösung gebracht wurden, wurden

sie vorher zweimal in Süßwasser und einmal in destilliertem Wasser gewaschen. Es wurden immer sechs Fische in je 500 ccm einer Lösung gebracht und jeden Tag die Zahl der überlebenden Fische festgestellt.

a) Entgiftung von NaBr.

Tabelle I gibt die Resultate einer Versuchsreihe über die Entgiftung von NaBr. Hierbei kommt es nicht allein auf die Zahl der überlebenden Tiere an, sondern auf die Zahl der von der Giftwirkung des Broms frei gebliebenen Tiere. Diese Giftwirkung macht sich dadurch bemerkbar, daß die Tiere auf der Seite liegen oder schwimmen und auf die geringste Erschütterung des Gefäßes wild aufspringen. Um nun die kranken von den unversehrten Tieren in der Tabelle zu unterscheiden, stellen wir die Zahl der überlebenden Tiere als eine Summe dar, wobei der erste Summand die unversehrten Tiere angibt, der zweite die Zahl der Tiere, die Vergiftungssymptome zeigen; so be-

Tabelle I.

	Zahl der nach 10 Tagen überlebenden Fische in			
	2	4	8	16
1	ccm $^m/_2$-NaBr in 100 ccm H_2O			
	0 + 3	0 + 3	0	0 + 4
	2	4	8	16
2	ccm $^m/_2$-NaBr + 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O			
	6	6	5 + 1	1 + 4
	2	4	8	16
3	ccm $^m/_2$-NaBr + 5 ccm $^m/_2$-NaCl in 100 ccm H_2O			
	6	5	4	0 + 6
	2	4	8	16
4	ccm $^m/_2$-NaBr + 2,2 ccm $^m/_2$-KBr + 1,5 ccm $^m/_2$-$CaBr_2$ in 100 ccm H_2O			
	1 + 1	3 + 2	0 + 2	0 + 1
	2	4	8	16
5	ccm $^m/_2$-NaBr in 100 ccm $^m/_8$-NaCl			
	6	6	4	3 + 2
	2	4	8	16
6	ccm $^m/_2$-NaBr in 100 ccm $^m/_8$-NaCl + KCl + $CaCl_2$			
	6	6	6	6

deutet beispielsweise 2 + 3, daß von den 5 überlebenden Tieren 2 normal und 3 vergiftet sind (auf der Seite liegen). 0 + 4 bedeutet, daß 4 Tiere überleben und vergiftet sind (auf der Seite liegen); 5 bedeutet, daß 5 Tiere überleben und alle normal sind. Wir geben die Resultate von sechs gleichzeitig angestellten Versuchsreihen in Tabelle I, und zwar dienen die Befunde am zehnten Versuchstage zum Vergleich.

In der reinen NaBr-Lösung (Reihe 1) unterliegen alle Tiere der Bromvergiftung, in der $^{m}/_{8}$-Lösung von NaCl + KCl + $CaCl_2$ (Reihe 6) bleiben alle Tiere von der Vergiftung verschont; keine andere Lösung zeigt ein so günstiges Resultat. Die nächstbeste Lösung war 5, die $^{m}/_{8}$-NaCl-Lösung. Der Umstand, daß in 16 ccm $^{m}/_{2}$-NaBr in 100 ccm $^{m}/_{8}$-NaCl ein Teil der Fische die Symptome der Bromvergiftung zeigte, beweist, daß nicht der ganze antagonistische Effekt dem Cl oder NaCl zugeschrieben werden darf, sondern daß der Zusatz von $CaCl_2$ + KCl nicht bedeutungslos ist.

Die Reihe 4 zeigt, daß der Zusatz von $CaBr_2$ + KBr nur eine sehr geringe entgiftende Wirkung hat, so daß die entgiftende Wirkung von $CaCl_2$ + KCl in Reihe 2 nicht auf das Ca und K allein bezogen werden darf, sondern auf die Salze $CaCl_2$ + KCl bezogen werden muß. Das ist theoretisch deshalb wichtig, weil es den Gedanken nahelegt, daß nicht Anionen, sondern die Chloridmoleküle für die antagonistische Wirkung in Betracht kommen. Ein Vergleich der Reihe 2 und 3 zeigt auch wieder, daß es nicht bloß auf die Anionen ankommt. In beiden Reihen ist der Zusatz an Cl-Ionen der gleiche, nur ist er in der einen Reihe (2) in der Form von $CaCl_2$ + KCl, in der anderen (3) in der Form von NaCl geliefert. $CaCl_2$ + KCl ist etwas günstiger als NaCl.

Wie schon in einer voraufgehenden Arbeit erwähnt war[1]), gelingt es weder mit $NaHCO_3$ noch mit Na_2SO_4, irgendwelche antagonistische Wirkungen gegen NaBr zu erzielen.

b) Die Entgiftung von NaJ.

Die Symptome bei der Vergiftung mit NaJ sind ähnlich wie die mit NaBr. Auch die Erscheinungen der Entgiftung sind ganz ähnlich, wie die Tabelle II zeigt.

[1]) Diese Zeitschr. **39**, 185, 1912.

Tabelle II.

	Zahl der nach 7 Tagen überlebenden Fische in			
	1	2	4	8
1	ccm $^m/_2$-NaJ in 100 ccm H_2O			
	0	0	0	0
	1	2	4	8
2	ccm $^m/_2$-NaJ + 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O			
	3 + 2	0 + 4	0 + 1	0
	1	2	4	8
3	ccm $^m/_2$-NaJ + 5 ccm $^m/_2$-NaCl in 100 ccm H_2O			
	6	5	0 + 4	0
	1	2	4	8
4	ccm $^m/_2$-NaJ in 100 ccm $^m/_8$-NaCl			
	4	5	0 + 4	0 + 1
	1	2	4	8
5	ccm $^m/_2$-NaJ in 100 ccm $^m/_8$-NaCl + KCl + $CaCl_2$			
	6	6	3 + 2	0 + 1

Wie im Falle von NaBr finden wir auch hier die kräftigste entgiftende Wirkung in der $^m/_8$-Lösung von NaCl + KCl + $CaCl_2$ (5). obwohl die $^m/_8$-Lösung von NaCl nahezu ebenso wirksam ist. Wir werden später sehen, daß auch hier die wesentlich entgiftende Wirkung von den Chlorsalzen ausgeht.

c) Die Entgiftung von $NaNO_3$.

Die Giftigkeit von $NaNO_3$ ist fast von der gleichen Größenordnung wie die von NaJ, und die Symptome sind ähnlich. Die Resultate einer Reihe von Versuchen werden in Tabelle III gegeben.

Wie in den voraufgehenden Versuchen ist die entgiftende Wirkung der Lösung von NaCl + KCl + $CaCl_2$ (5) größer als die irgendeiner anderen Kombination von entgiftenden Salzen. Am nächsten kommt ihr die entgiftende Wirkung einer $^m/_8$-NaCl-Lösung (4). $Ca(NO_3)_2$ + KNO_3 haben so gut wie keine entgiftende Wirkung, während KCl + $CaCl_2$ eine ziemlich kräftige entgiftende Wirkung ausübt, was wieder zeigt, daß die entgiftende Wirkung nicht von den Kationen Ca + K ausgeht, sondern von den Molekülen $CaCl_2$ und KCl. Na_2SO_4 und Natriumacetat hatten keine entgiftende Wirkung auf Natriumnitrat.

Tabelle III.

	Zahl der überlebenden Fische nach 4 Tagen in			
	5	10	15	20
1	ccm $^{m}/_{2}$-$NaNO_3$ in 100 ccm H_2O			
	0	0	0	0
	5	10	15	20
2	ccm $^{m}/_{2}$-$NaNO_3$ + 2,2 ccm $^{m}/_{2}$-KCl + 1,5 ccm $^{m}/_{2}$-$CaCl_2$ in 100 ccm H_2O			
	6	5	0 + 1	0
	5	10	15	20
3	ccm $^{m}/_{2}$-$NaNO_3$ + 2,2 ccm $^{m}/_{2}$-KNO_3 + 15 ccm $^{m}/_{2}$-$Ca(NO_3)_2$ in 100 ccm H_2O			
	0 + 3	0	0	0
	5	10	15	20
4	ccm $^{m}/_{2}$-$NaNO_3$ in 100 ccm $^{m}/_{8}$-$NaCl$			
	6	6	1	0
	5	10	15	20
5	ccm $^{m}/_{2}$-$NaNO_3$ in 100 ccm $^{m}/_{8}$-$NaCl$ + KCl + $CaCl_2$			
	6	6	6	0 + 4

d) Die Entgiftung von Natriumrhodanat.

Natriumrhodanat ist erheblich giftiger als die bisher untersuchten Salze. Es war daher nötig, mit niedrigeren Konzentrationen zu arbeiten, um die Überlegenheit der entgiftenden Wirkung der Ringer-Lösung über die übrigen Lösungen zu zeigen.

Tabelle IV.

	Zahl der überlebenden Fische nach 2 Tagen in		
	4	8	12
1	ccm $^{m}/_{8}$-$NaCNS$ in 100 ccm H_2O		
	0	0	0
	4	8	12
2	ccm $^{m}/_{8}$-$NaCNS$ + 2,2 ccm $^{m}/_{2}$-KCl + 1,5 ccm $^{m}/_{2}$-$CaCl_2$ in 100 ccm H_2O		
	1	0	0
	4	8	12
3	ccm $^{m}/_{8}$-$NaCNS$ + 5 ccm $^{m}/_{2}$-$NaCl$ in 100 ccm H_2O		
	0	0	0
	4	8	12
4	ccm $^{m}/_{8}$-$NaCN$ in 100 ccm $^{m}/_{8}$-$NaCl$		
	2 + 2	0	0
	4	8	12
5	ccm $^{m}/_{8}$-$NaCNS$ in 100 ccm $^{m}/_{8}$-$NaCl$ + KCl + $CaCl_2$		
	6	5	0

Wie in allen voraufgehenden Versuchen ist auch hier wieder die entgiftende Wirkung der Lösung von NaCl + KCl + $CaCl_2$ größer als die irgendeiner anderen Kombination von Salzen. In der $^m/_8$-Lösung von NaCl + KCl + $CaCl_2$ mit NaCNS lebten die Fische mehrere Wochen lang.

e) Die Entgiftung von essigsaurem Natrium.

Essigsaures Natrium ist relativ ungiftig und deshalb wurden höhere Konzentrationen zur Anwendung gebracht. Es findet in diesen Lösungen aber rasch eine kräftige Entwicklung von Bakterien statt und es ist daher nötig, nur mit relativ kurzen Zeiträumen zu operieren.

Tabelle V.

	Zahl der überlebenden Fische nach 5 Tagen in			
	30	40	50	60
1	ccm $^m/_2$-$NaCH_3COO$ in 100 ccm H_2O			
	1	0	0	0
	30	40	50	60
2	ccm $^m/_2$-$NaCH_3COO$ + 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O			
	6	3	0	0
	30	40	50	60
3	ccm $^m/_2$-$NaCH_3COO$ + 2,2 ccm $^m/_2$-KCH_3COO + 1,5 ccm $^m/_2$-$Ca(CH_3COO)_2$ in 100 ccm H_2O			
	4	0	0	0
	30	40	50	60
4	ccm $^m/_2$-$NaCH_3COO$ + 5 ccm $^m/_2$-NaCl in 100 ccm H_2O			
	6	4	0	0
	30	40	50	60
5	ccm $^m/_2$-$NaCH_3COO$ in 100 ccm $^m/_8$-NaCl			
	0	0	0	0
	30	40	50	60
6	ccm $^m/_2$-$NaCH_3COO$ in 100 ccm $^m/_8$-NaCl + KCl + $CaCl_2$			
	6	6	2	0

Auch hier finden wir die höchste Toleranz für essigsaures Natrium in der Lösung von NaCl + KCl + $CaCl_2$. Es könnte auf den ersten Blick befremden, daß eine $^m/_8$-NaCl-Lösung eine so geringe entgiftende Wirkung auf essigsaures Natrium hat, während in den bisher betrachteten Fällen beispielsweise

bei der Entgiftung von NaJ oder NaBr die entgiftende Wirkung von $^{m}/_{8}$-NaCl eine recht kräftige war. Das Rätsel findet wohl seine Lösung in der Tatsache, daß wegen der relativ geringen Giftigkeit von essigsaurem Natrium recht hohe Konzentrationen des letzteren Salzes gewählt werden mußten, wodurch die Konzentration von Na die giftige Grenze fast erreichte. Wurde nun die Lösung des essigsauren Na-Acetat in $^{m}/_{8}$-NaCl gemacht, so trat dieselbe Situation ein, die wir in den früheren Arbeiten für zu hohe Konzentrationen von NaCl schilderten: nämlich eine solche Lösung ist ohne Zusatz von $CaCl_2$ giftig. Man muß bei der Entgiftung nicht nur die Natur der Salze, sondern auch ihre Konzentrationen berücksichtigen.

Wir verstehen es nun auch, daß ein kleinerer Betrag von NaCl, nämlich 5 ccm $^{m}/_{2}$-NaCl in 100 ccm H_2O, eine deutliche entgiftende Wirkung ausübt, wie 4 zeigt und daß diese Wirkung ebenso stark ist wie die durch Zusatz von $CaCl_2 + KCl$ erzielte, was darauf hinweist, daß die Chloridmoleküle die wesentliche entgiftende Wirkung ausüben. $KCH_3COO + Ca(CH_3COO)_2$ üben fast keine entgiftende Wirkung aus, was nach dem Gesagten ja zu erwarten war.

Wir müssen uns deshalb folgende Vorstellung über die entgiftende Wirkung der $^{m}/_{8}$-Lösung von $NaCl + KCl + CaCl_2$ in diesem Falle bilden. Die wesentlich entgiftende Wirkung geht vom NaCl aus. Das $CaCl_2 + KCl$ dient zur Entgiftung der Na-Salze dieser Lösung, deren Konzentrationen die Giftgrenze übersteigt.

f) Die Entgiftung von buttersaurem Natrium.

Um die vorhin aufgestellte Behauptung zu prüfen, daß die $^{m}/_{8}$-NaCl-Lösung nur deshalb das essigsaure Natrium nicht zu entgiften imstande war, weil das letztere wegen seiner geringen Giftigkeit in zu hohen Konzentrationen zur Anwendung kommen mußte, wurden Versuche mit buttersaurem Natrium angestellt. Das letztere ist viel giftiger als das essigsaure Natrium und konnte deshalb in viel geringeren Konzentrationen zur Anwendung kommen. Es war deshalb zu erwarten, daß dieses Salz durch eine $^{m}/_{8}$-NaCl-Lösung wenigstens zum Teil entgiftet werden könne, was auch in der Tat zutraf.

Tabelle VI.

	Zahl der überlebenden Fische nach 6 Tagen in			
	4	6	8	12
1	ccm $^m/_2$-Nabutyrat in 100 ccm H_2O			
	2	4	0	0
	4	6	8	12
2	ccm $^m/_2$-Nabutyrat + 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O			
	3	6	5	6
	4	6	8	12
3	ccm $^m/_2$-Nabutyrat + 5 ccm $^m/_2$-NaCl in 100 ccm H_2O			
	4	4	5	3
	4	6	8	12
4	ccm $^m/_2$-Nabutyrat in 100 ccm $^m/_8$-NaCl			
	6	6	4	0
	4	6	8	12
5	ccm $^m/_2$-Nabutyrat in 100 ccm $^m/_8$-NaCl + KCl + $CaCl_2$			
	6	6	6	4

Der Umstand, daß der Zusatz von 5 ccm $^m/_2$-NaCl (3) fast so günstig wirkt, wie der Zusatz von 2.2 ccm $^m/_2$-KCl + 1.5 ccm $^m/_2$-$CaCl_2$ zeigt, daß die entgiftende Wirkung dieser Stoffe zum großen Teile ihrem Gehalt an Cl zuzuschreiben ist.

g) Die Entgiftung von Natriumsulfat.

Na_2SO_4 ist im Seewasser enthalten und kann deshalb nicht als artfremdes Salz für die Fische angesehen werden. Die Versuche mit Natriumsulfat bringen einen neuen theoretisch wichtigen Umstand in die Analyse dieser Vorgänge. In allen bisher erwähnten Versuchen mit Natriumsalzen fiel die entgiftende Wirkung hauptsächlich den Chloriden zu. Das ist vielleicht auch bei Na_2SO_4 der Fall. Aber Na_2SO_4 ist relativ ungiftig und muß deshalb in diesen Versuchen in hohen Konzentrationen angewendet werden; das wird dieselbe Komplikation bedingen, der wir schon im Falle von $NaCH_3COO$ begegneten, daß eine $^m/_8$-NaCl-Lösung nur dann entgiftend wirkt, wenn KCl und $CaCl_2$ in der Lösung sind. Zweitens aber gehört SO_4 zu den calciumfällenden Ionen. Na_2SO_4 wirkt also in einer spezifisch anderen Weise als alle die bisher betrachteten Salze, und so dürfen wir auch a priori erwarten, daß Ca für die Entgiftung

von Na_2SO_4 eine viel größere Rolle spielt als in den bisher erwähnten Versuchen. Alles das trifft zu.

Es sei nebenbei erwähnt, daß, wenn man die Fische in die Lösung irgendeines calciumfällenden Salzes bringt, z. B. Natriumoxalat, Natriumtartrat und Na_2SO_4, oft (aber nicht immer) Blutungen aus den Kiemen stattfinden. Es handelt sich offenbar um Erhöhung der Durchgängigkeit der Blutgefäße, vielleicht auch um Verringerung der Gerinnfähigkeit des Blutes, beides infolge der Entziehung des Ca aus der Oberflächenlamelle der Gefäße. Die in Tabelle VII wiedergegebenen Versuche wurden wie immer gleichzeitig angestellt.

Tabelle VII.

	Zahl der überlebenden Fische nach 3 Tagen in			
	35	40	45	50
1	ccm $^m/_2$-Na_2SO_4 in 100 ccm H_2O			
	0	0	0	0
	35	40	45	50
2	ccm $^m/_2$-Na_2SO_4 + 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$ in 100 ccm H_2O			
	6	6	5	4
	35	40	45	50[1]
3	ccm $^m/_2$-Na_2SO_4 + 2,2 ccm $^m/_2$-K_2SO_4 + 1,5 ccm $^m/_2$-$CaSO_4$ in 100 ccm H_2O			
	0	1	0	0
	35	40	45	50
4	ccm $^m/_2$-Na_2SO_4 + 5 ccm $^m/_2$-NaCl in 100 ccm H_2O			
	0	0	0	0
	35	40	45	50
5	ccm $^m/_2$-Na_2SO_4 in 100 ccm $^m/_8$-NaCl			
	0	0	0	0
	35	40	45	50
6	ccm Na_2SO_4 in 100 ccm $^m/_8$-NaCl + KCl + $CaCl_2$			
	6	5	1	0

Diese Resultate sind charakteristisch. Die stärkste entgiftende Wirkung finden wir in Lösung 2 bei Zusatz von 2,2 ccm $^m/_2$-KCl + 1,5 ccm $^m/_2$-$CaCl_2$. Kontrollversuche zeigten.

[1]) Der Kürze halber ist der Ausdruck $^m/_2$-$CaSO_4$ gebraucht. In Wirklichkeit war natürlich das Äquivalent von 1,5 ccm $^m/_2$-$CaSO_4$ in 100 ccm der Lösung gelöst.

daß das $CaCl_2$ der wesentlich wirksame Bestandteil ist. Es ist nun von großem Interesse, daß $CaSO_4$ fast unwirksam ist und nur $CaCl_2$ wirksam ist, wie ein Vergleich von 2 mit 3 zeigt. Andererseits ist der Zusatz von 5 ccm $^m/_2$-NaCl ganz unwirksam. Das weist meines Erachtens darauf hin, daß die entgiftende Wirkung von Salzen und nicht von Ionen ausgeht. $CaCl_2$ entgiftet; $CaSO_4$ entgiftet nicht! Daß eine $^m/_8$-Lösung von NaCl + $CaCl_2$ + KCl nicht so günstig wirkt wie der Zusatz von 2,2 ccm KCl + 1,5 ccm $CaCl_2$, liegt wohl daran, daß die Na-Konzentration zu hoch war. Es kann aber auch ein Zufall im Spiele sein.

Es gelang ferner auch zu zeigen, daß die entgiftende Wirkung einer Lösung von $^m/_8$-NaCl + KCl + $CaCl_2$ auf LiCl und NH_4Cl ein Maximum war. Wir wollen aber hierauf in dieser Arbeit nicht eingehen.

Wir dürfen demnach wohl schließen, daß eine $^m/_8$-Lösung von NaCl + KCl + $CaCl_2$ in dem Verhältnis, in dem diese Salze im Seewasser enthalten sind, eine größere Menge von Salzen mit artfremden Ionen zu entgiften vermag, als irgendeine andere Kombination. Vielleicht würde eine Lösung von NaCl + KCl + $CaCl_2$ in einer etwas höheren Konzentration als $^m/_3$ eine noch etwas größere entgiftende Wirkung besitzen. Das ist hier nicht untersucht worden.

II.

Wir haben in früheren Arbeiten den Begriff des Entgiftungskoeffizienten eingeführt; darunter verstehen wir den Quotienten der Konzentration des giftigen in die zur Entgiftung eben ausreichende Konzentration des entgiftenden Salzes. Dabei sprechen wir von entgiftender Wirkung, wenn die Fische von den Vergiftungssymptomen frei bleiben. Zur

Tabelle VIII.

Nach Tagen	Zahl der überlebenden Fische in 6 ccm $^m/_2$-$NaNO_3$ + ccm $^m/_4$-$CaCl_2$ in 100 ccm H_2O								
	1	2	4	6	8	16	25	36	48
3	4 + 1	6	6	6	6	6	6	4	3
6	1 + 3	2 + 4	6	6	5	6	5	3	3
8	0 + 4	2 + 4	5	6	5	6	5	2	1
12	0 + 4	0 + 6	5	6	4	6	2	0	0

Illustration mögen neben den in unseren früheren Arbeiten mitgeteilten Versuchen auch die folgenden einen Platz finden. Wie erwähnt, wurden stets 6 Fische am Anfang des Versuches in je 500 ccm der Lösung gebracht und die Zahl der überlebenden Fische jeden Tag festgestellt.

In Tabelle VIII haben wir ein ziemlich bestimmtes Resultat. Der Zusatz von 4 ccm $^m/_4$-$CaCl_2$ und darüber verhinderte während der Versuchsdauer den Eintritt der Nitratvergiftung (Verlust des Gleichgewichtes der Fische), und wir dürfen deshalb sagen, daß 4 ccm $^m/_4$-$CaCl_2$ zur Hemmung der Giftwirkung von 6 ccm $^m/_2$-$NaNO_3$ ausreichen. Der Entgiftungskoeffizient $NaNO_3/CaCl_2$ ist deshalb $= 3$. Der Zusatz von 36 oder mehr ccm $^m/_4$-$CaCl_2$ wirkt durch den Überschuß von $CaCl_2$ schädlich.

Wir fügen nun zum Vergleich einen gleichzeitig angestellten Entgiftungsversuch von 6 ccm $^m/_2$-$NaNO_3$ durch NaCl bei.

Tabelle IX.

Nach Tagen	Zahl der überlebenden Fische in 6 ccm $^m/_2$-$NaNO_3$ + ccm in 100 ccm $^m/_2$-NaCl in 100 ccm H_2O						
	0	2	4	6	8	16	25
3	1 + 2	1 + 2	4 + 1	4 + 1	3 + 2	5	6
6	0 + 1	1 + 1	2	0 + 1	4	5	6
8	0	0	1	1	3	5	5
12			0	1	1	4	0

Vergleichen wir Tabelle IX mit Tabelle VIII, so fällt zunächst auf, daß viel mehr Fische in der NaCl-Reihe (Tabelle IX) sterben als in der $CaCl_2$-Reihe (Tabelle VIII). Die Entgiftung durch $CaCl_2$ ist also eine vollständigere als die durch NaCl. Wenn wir die Entgiftung darauf zurückführen, daß durch Zusatz des entgiftenden Salzes die Oberfläche (etwa die Kiemen der Fische) für beide Salze undurchgängig wird, so müssen wir sagen, daß $CaCl_2$ die „Gerbung" der Oberflächenlamelle besser besorgt als NaCl. Auch die Grenze, bei der die Entgiftung durch NaCl herbeigeführt wird, ist in diesem Falle nicht so scharf bestimmbar wie im Falle der Entgiftung durch $CaCl_2$. Es unterliegt keinem Zweifel, daß 16 ccm $^m/_2$-NaCl für die Entgiftung ausreichen. Wir dürfen aber wohl den Wert 8 ccm $^m/_2$-NaCl als die Grenze annehmen. Das wird durch andere Versuchsreihen bestätigt, die hier wohl nicht mitgeteilt

zu werden brauchen. Wir würden danach den Entgiftungskoeffizienten $NaNO_3/NaCl = {}^6/_8$ ansehen.

Zwei Versuchsreihen über die Entgiftung von 4 ccm $NaNO_3$ in 100 ccm H_2O durch NaCl und $CaCl_2$ ergaben die folgenden Werte:

4 ccm $NaNO_3$ entgift. durch 4 ccm $^m/_2$-NaCl E.-K. $NaNO_3/NaCl = 1$
4 „ $NaNO_3$ „ „ 2 „ $^m/_4$-$CaCl_2$ „ $NaNO_3/CaCl_2 = 4$

Eine Versuchsreihe mit 10 ccm $NaNO_3$ in 100 ccm H_2O ergab die folgenden Entgiftungskoeffizienten:

10 ccm $NaNO_3$ entgift. d. 8 ccm $^m/_2$-NaCl E.-K. $NaNO_3/NaCl = 1.25$
10 „ $NaNO_3$ „ „ 4 „ $^m/_2$-$CaCl_2$ „ $NaNO_3/CaCl_2 = 2$

15 ccm $^m/_2$-$NaNO_3$ in 100 ccm H_2O konnten überhaupt durch keine Quantität von NaCl oder $CaCl_2$ mehr entgiftet werden. Dieses Bestehen einer oberen Grenze des giftigen Salzes, oberhalb der keine Entgiftung mehr möglich ist, ist charakteristisch für alle diese Versuche, worauf schon bei Gelegenheit der Entgiftung von KCl durch NaCl hingewiesen wurde. Versuche, das $NaNO_3$ durch $Ca(NO_3)_2$ zu entgiften, gelangen nicht; ebensowenig gelang es, irgendwelche entgiftende Wirkung durch Na_2SO_4 zu erzielen. Wir dürfen daraus wohl schließen, erstens daß nur Chloride zur Entgiftung von $NaNO_3$ geeignet sind; und zweitens, daß die entgiftende Wirkung von $CaCl_2$ derjenigen von NaCl überlegen ist; ein Resultat, das sich überall bestätigt, wo nur niedrige Konzentrationen beider Salze in Betracht kommen, worauf wir schon in einer voraufgehenden Abhandlung hingewiesen haben.

Für die Ermittlung der Entgiftungskoeffizienten von NaJ haben wir nur Versuchsreihen für 2 ccm $^m/_2$-NaJ in 100 ccm H_2O. In einem Falle wurde NaCl, im anderen $CaCl_2$ zur Entgiftung verwendet. Tabellen X und XI dürfen als Beispiel dienen.

Tabelle X.

Nach Tagen	Zahl der überlebenden Fische in 2 ccm $^m/_2$-NaJ + ccm $^m/_2$-NaCl in 100 ccm H_2O								
	0	1	2	3	4	6	8	10	15
3	0	0	2	2	2	4	6	4	6
6			0 + 1	1 + 1	0	3	4	4	3
11			0 + 1	0		3	3	4	3

Tabelle XI.

Nach Tagen	Zahl der überlebenden Fische in 2 ccm $^m/_2$ NaJ — ccm $^m/_4$-$CaCl_2$ in 100 ccm H_2O							
	1	2	3	4	6	8	10	15
3	3	4	4 + 1	6	6	6	5	6
6	1	4	4 + 1	5 + 1	6	6	4	6
11	0 + 1	4	5	6	6	6	4	6

Die Zahl der überlebenden Fische ist wieder größer bei der Entgiftung durch $CaCl_2$ als bei der Entgiftung durch NaCl. Als die Grenze für die Entgiftung von 2 ccm $^m/_2$-NaJ in 100 ccm H_2O können wir 6 ccm $^m/_2$-NaCl und etwa 4 bis 6 ccm $^m/_4$-$CaCl_2$ annehmen. Der Entgiftungskoeffizient ist also für NaJ/NaCl etwa $^1/_3$; für NaJ/$CaCl_2$ etwa 1 bis $^2/_3$. Es ist beachtenswert, daß in dem Versuche mit $CaCl_2$ als entgiftendes Salz in den Lösungen mit wenig $CaCl_2$ die Fische in den ersten Tagen Gleichgewichtsstörungen zeigen, die später wieder verschwinden. Hier handelt es sich wohl darum, daß allmählich durch Einwirkung von Ca etwas HJ gebildet wird, die in einer für die Fische unschädlichen Weise an der Oberfläche der Fische gebunden wird; oder daß durch Bildung von freiem Jod allmählich die Konzentration des NaJ vermindert wird, so daß nun die anfänglich unzulängliche Menge von $CaCl_2$ für die Entgiftung ausreicht.

Im Falle von NaCNS wurde gefunden, daß 4 ccm $^m/_8$-NaCNS durch 36 ccm $^m/_2$-NaCl und durch 8 ccm $^m/_2$-$CaCl_2$ entgiftet werden. Auch hier blieben bei Anwendung von $CaCl_2$ viel mehr Fische am Leben als bei Anwendung von NaCl.

Wie schon erwähnt, verhält sich Na_2SO_4 abweichend von diesen Salzen in doppelter Hinsicht: Erstens wird es erst giftig in viel höheren Konzentrationen und zweitens konkurriert es um das Ca, von dem wir annehmen, daß es an der Oberfläche der Zellen chemisch gebunden ist. Es gelingt nun so gut wie gar nicht, Na_2SO_4 durch NaCl zu entgiften, was wir ja schon früher diskutiert haben. Dagegen gelingt die Entgiftung von Na_2SO_4 durch $CaCl_2$ und $MgCl_2$. Wir wollen einige Belege hierfür geben.

Tabelle XII.

Nach Tagen	Zahl der überlebenden Fische in 25 ccm $^m/_2$-Na_2SO_4 + ccm $^m/_2$-NaCl in 100 ccm H_2O								
	0	1	2	4	8	16	20	25	30
2	0	0	0	0	0	1	0	0	0
3						0			

In einer Reihe dieser Lösung fanden Blutungen aus den Kiemen statt. Gleichzeitig wurde der folgende Versuch angestellt.

Tabelle XIII.

Nach Tagen	Zahl der überlebenden Fische in 25 ccm $^m/_2$-Na_2SO_4 + ccm $^m/_{50}$-$CaCl_2$ in 100 ccm H_2O							
	1	2	4	8	16	20	25	30
2	2	4	6	6	6	5	5	6
3	1	1	3	2	4	3	5	5
7	0	0	1	0	2	3	4	4

In keinem Versuche der Calciumreihe kam es zu Blutungen. Ein Vergleich der Tabellen zeigt, daß NaCl keine Entgiftung herbeiführt, während $CaCl_2$ in dieser Hinsicht äußerst wirksam ist. Auch durch $MgCl_2$ läßt sich die Giftwirkung von Na_2SO_4 hemmen, nur ist hierfür eine viel größere Konzentration von $MgCl_2$ als von $CaCl_2$ erforderlich, was ja den für die Entgiftung von NaCl und KCl gewonnenen Erfahrungen entspricht.

Tabelle XIV.

Nach Tagen	Zahl der überlebenden Fische in 30 ccm $^m/_2$-Na_2SO_4 + ccm $^m/_4$-$MgCl_2$ in 100 ccm H_2O										
	0	0,5	1,0	2,0	3,0	4,0	5,0	7,5	10,0	15,0	20,0
1	0	2	3	5	4	4	6	6	6	6	6
4		0	0	0	0	1	4	3	6	5	5
7						1	1	0	6	4	5

Man kann in diesem Falle die Grenze für die Hemmung der Giftwirkung bei 4,0 ccm $^m/_4$-$MgCl_2$ setzen. Ein gleichzeitig angestellter Versuch mit $CaCl_2$ ergab dieselbe Grenze bei 5.0 ccm $^m/_{100}$-$CaCl_2$. Die entgiftende Wirkung von $CaCl_2$ ist demnach etwa 20mal so groß wie die von $MgCl_2$.

Wir kommen also zu dem Resultat, daß die Giftwirkung von nichtchlorhaltigen Natriumsalzen durch Chloride, und zwar

durch NaCl, $CaCl_2$ und $MgCl_2$ gehemmt werden kann. Die Calciumsalze haben durchweg eine stärkere gifthemmende Wirkung als die Natriumsalze. Dieser Unterschied ist besonders groß bei Na_2SO_4.

III.

Wir haben wiederholt in dieser Arbeit auf die Tatsache hingewiesen, daß $CaCl_2$ eine stärkere entgiftende Wirkung hat als andere Calciumsalze. Wenn man das Äquivalent von 1,5 ccm $^m/_2$-$CaCl_2$ in der Form von $CaSO_4$ zu Na_2SO_4 zufügt, so wird nur eine geringe Verzögerung der Giftwirkung von Na_2SO_4 beobachtet. In 25 ccm $^m/_2$-Na_2SO_4 + Äquivalent 1,5 ccm $^m/_2$-$CaSO_4$ in 100 ccm H_2O leben die Fische etwa 1 Tag länger als in 25 ccm $^m/_2$-Na_2SO_4 in 100 ccm H_2O. Es gelingt auch nicht ein besseres Resultat zu erzielen, wenn man $CaSO_4$ in irgendeinem anderen Verhältnis zusetzt. Setzt man aber 0,4 ccm bis 3,0 ccm $^m/_{20}$-$CaCl_2$ zu 25 ccm $^m/_2$-Na_2SO_4 in 100 ccm H_2O zu, so bleiben die meisten oder alle Fische wochenlang am Leben. In diesem Falle hängt also die entgiftende Wirkung fast gar nicht von dem Ca-Ion, sondern von dem Molekül $CaCl_2$ ab. Es war nun von Interesse zu untersuchen, ob andere Calciumsalze die Giftwirkung von Na_2SO_4 zu hemmen vermögen. Es wurde essigsaures Calcium, $CaBr_2$ und $Ca(NO_3)_2$ angewendet. Alle vermochten die Giftwirkung von Na_2SO_4 nur sehr wenig zu hemmen, obwohl die Fische in den reinen Lösungen dieser Calciumsalze, in den Konzentrationen, die in diesen Versuchen benutzt wurden, beliebig lange leben. Wir sahen, daß $MgCl_2$ imstande ist, die Giftwirkung von Na_2SO_4 etwas zu hemmen. Wir stellten eine Versuchsreihe mit $MgSO_4$ an und fanden, daß dieser Stoff fast wirkungslos ist. Tabelle XV gibt die Resultate.

Hierzu ist folgendes zu bemerken: Während die Lösungen mit 16 ccm oder mehr $^m/_{20}$-$CaCl_2$ völlig entgiftet waren und blieben, und selbst die Lösungen mit weniger $CaCl_2$ teilweise entgiftet waren, war in den Lösungen, in denen $CaBr_2$ oder $Ca(NO_3)_2$ zugesetzt war, so gut wie keine entgiftende Wirkung vorhanden. Nur das essigsaure Calcium (4) hat eine schwache gifthemmende Wirkung. Nach 6 Tagen waren aber auch in dieser Reihe alle Fische bis auf einen tot, während in Reihe 1

Tabelle XV.

	Zahl der überlebenden Fische nach 4 Tagen in								
	25 ccm $^m/_2$-Na_2SO_4 +	0	2	4	8	16	20	30	
1	ccm $^m/_{20}$-$CaCl_2$ in 100 ccm H_2O	0	2	3	2	6	6	5	
	25 ccm $^m/_2$-Na_2SO_4 +	0	2	4	8	16	20	30	
2	ccm $^m/_{20}$-$CaBr_2$ in 100 ccm H_2O	0	1	0	0	0	0+1	0	
	25 ccm $^m/_2$-Na_2SO_4 +	0	2	4	8	16	20	30	
3	ccm $^m/_{20}$-$Ca(NO_3)_2$ in 100 ccm H_2O	0	0	0	0	0	0	0	
	25 ccm $^m/_2$-Na_2SO_4 +	0	2	4	8	16	20	30	
4	ccm $^m/_{20}$-$Ca(CH_3COO)_2$ in 100 ccm H_2O	0	1	0	0	0	2	1	
	25 ccm $^m/_2$-Na_2SO_4 +	0	2	4	8	16	20	30	40	50
5	ccm $^m/_2$-$MgSO_4$ in 100 ccm H_2O	0	0	0	0	0	1	0	0	0

mit $CaCl_2$ noch 18 Fische am Leben waren. Auch $MgSO_4$ hat nur eine sehr schwache hemmende Wirkung auf Na_2SO_4. Wir haben also folgende drei Gruppen von Tatsachen: 1. Eine an sich giftige Lösung von Na_2SO_4 wird durch Zusatz von einer kleinen Quantität $CaCl_2$ und mit einer etwas größeren von $MgCl_2$ entgiftet. 2. Die Giftigkeit derselben Na_2SO_4-Lösung wird durch eine NaCl-Lösung mit demselben oder mit geringerem oder größerem Gehalt an Cl nicht verringert. 3. Die Giftigkeit derselben NaCl-Lösung wird durch $CaSO_4$, $CaBr_2$, $Ca(NO_3)_2$, $Ca(CH_3COO)_2$ und $MgSO_4$ nicht oder fast nicht verringert. Daraus folgt, daß die antagonistische Wirkung von $CaCl_2$ auf Na_2SO_4 durch das Salz $CaCl_2$ und nicht durch das Ca-Ion oder das Cl-Ion bestimmt ist.

Eine ähnliche Versuchsreihe wurde für die Entgiftung von 4 ccm $NaNO_3$ durchgeführt. Auch hier zeigte es sich, daß $CaCl_2$ eine kräftige entgiftende Wirkung hat, daß aber $Ca(NO_3)_2$ und essigsaures Calcium fast keine entgiftende Wirkung besitzen.

2 Tage später waren in Reihe 3 alle Fische tot, während sie in Reihe 1 meist beliebig lange, d. h. wochenlang, weiter leben.

Aber in diesem Falle haben wir die Komplikation, daß auch NaCl in geringer Konzentration entgiftend wirkt, so daß

Tabelle XVI.

	Zahl der überlebenden Fische nach 3 Tagen in							
	4 ccm $^{m}/_{2}$-$NaNO_3$ +	0	1	2	4	8	16	20	30
1	ccm $^{m}/_{20}$-$CaCl_2$ in 100 ccm H_2O	0	0	2	3	6	5	3	6
	4 ccm $^{m}/_{2}$-$NaNO_3$ +		1	2	4	8	16	20	30
2	ccm $^{m}/_{20}$-$Ca(CH_3COO)_2$ in 100 ccm H_2O		0	0	0	0	0	0	0
	4 ccm $^{m}/_{2}$-$NaNO_3$ +		1	2	4	8	16	20	30
3	ccm $^{m}/_{2}$-$Na(NO_3)_2$ in 100 ccm H_2O		1	0	0	0	1+1	0	0

in diesem Falle die entgiftende Wirkung dem Cl-Ion zugeschrieben werden dürfte.

Weitere Beispiele derselben Art finden sich in Tabelle I und V. Es gibt also jedenfalls Fälle, in denen wir mit Sicherheit behaupten können, daß die entgiftende Wirkung eines Calciumsalzes nicht von einem Ion, sondern vom Molekül ausgeht. Wir dürfen aber den Fall nicht verallgemeinern, da wir früher sahen, daß KCl auch durch Na_2SO_4 entgiftet werden kann, und zwar ist das letztere 2mal so wirksam wie eine äquimolekulare Lösung von NaCl. In diesem Falle ging also die entgiftende Wirkung wohl von dem Na-Ion aus; wenigstens ist nicht bewiesen oder beweisbar, daß sie vom Salzmolekül ausgeht.

Ich fand, daß NaJ durch NaCl, aber weder durch Na_2SO_4 noch durch $NaNO_3$ oder $NaCH_3COO$ entgiftet werden kann. Ob aber das Cl-Ion und nicht das NaCl die Entgiftung bewirkt, läßt sich nicht entscheiden. LiCl und NH_4Cl sind schon in so kleinen Dosen giftig, daß dieselben für diese Zwecke nicht verwertbar sind. Es scheint aber, daß NH_4Cl eine etwas stärker entgiftende Wirkung auf NaJ hat als das viel ungiftigere $NaCH_3COO$.

IV.

Auf Grund früherer Arbeiten war der Autor zu der Annahme gelangt, daß die antagonistische Wirkung der Elektrolyte zum Teil wenigtens darauf beruht, daß die antagonistischen Elektrolyte sich gegenseitig am Eindringen in die Zellen oder die Organismen hindern; und zwar dadurch, daß sie die Ober-

flächenlamelle füreinander undurchgängiger machen. Diese Änderung haben wir als „Gerbung" bezeichnet, wobei wir jedoch im Auge behalten müssen, daß der eigentlichen Gerbung ein irreversibler Prozeß zugrunde liegt, während es sich bei der Beeinflussung der Oberflächenlamelle lebender Zellen durch Salze oft wenigstens um reversible Änderungen handelt.

Diese Ansichten führten logischerweise zu der Annahme, daß die Mischung von $NaCl + KCl + CaCl_2$ in dem Verhältnis, in dem diese Salze im Seewasser vorhanden sind, deshalb für die Erhaltung des Lebens der Zelle so günstig ist, weil sie der Oberflächenlamelle diejenige physikalische Beschaffenheit erteilt, bei der sie am dauerhaftesten und zugleich für Elektrolyte am undurchgängigsten ist. Um diese letztere Ansicht zu prüfen, waren die hier geschilderten Versuche unternommen worden. Wenn wirklich die Mischung von $NaCl + KCl + CaCl_2$ die Zellen am undurchgängigsten für Elektrolyte macht, so sollte sich das auch u. a. darin zeigen, daß Salze mit artfremden Ionen dann am langsamsten in die Tiere oder die Zellen eindringen, wenn die artfremden Elektrolyte einer Mischung von $NaCl + KCl + CaCl_2$ zugefügt werden, als wenn sie irgendeiner anderen Lösung von Salzen zugefügt werden. Das müßte sich darin zeigen, daß die Tiere in einer Mischung von $NaCl + KCl + CaCl_2$ eine höhere Dosis dieser giftigen Salze ertragen sollten als in irgendeiner anderen Lösung oder Mischung von Salzen. Das traf nun in unseren Versuchen an Fundulus zu. Ein Versuch dieser Art kann nur bei solchen Organismen Beweiskraft beanspruchen, die von dem osmotischen Druck oder der Zusammensetzung des umgebenden Mediums in weiten Grenzen unabhängig sind. Denn sonst würde ja der Einwand naheliegen, daß auch bei positivem Ausfall die Versuche nichts beweisen, da die Tiere ohnedies nur in einer Mischung von $NaCl + KCl + CaCl_2$ leben können. Die Tiere, die wir zu diesen Versuchen gewählt haben, bilden fast ein Unikum in der Biologie, indem sie hinreichend klein sind, um zu Versuchen benutzbar zu sein, in hinreichend großen Mengen leicht erhalten werden können und in weiten Grenzen sowohl vom osmotischen Druck wie von der Zusammensetzung des Mediums unabhängig sind. Wie schon in den früheren Arbeiten erwähnt wurde, leben diese Fische beliebig lange in einer Lösung von 5 ccm

$^{m}/_{2}$-NaCl in 100 ccm H_2O oder in einer Lösung von 1,5 ccm $^{m}/_{2}$-$CaCl_2$ + 2,2 ccm $^{m}/_{2}$-KCl in 100 ccm H_2O oder in reinen Lösungen von $MgCl_2$ oder $CaCl_2$, wenn dieselben keine zu hohe Konzentration erreichen, oder in Lösungen von $Ca(NO_3)_2$ oder $CaBr_2$ von niedriger Konzentration; kurz in all den Lösungen, die in diesen Versuchen zur Entgiftung benutzt wurden. Man kann also nicht sagen, daß es sich hier um Versuchstiere handelt, die nur an eine Mischung von NaCl + KCl + $CaCl_2$ angepaßt seien.

Ein solcher Einwand würde nun nun auch durch die Versuche des zweiten Abschnittes widerlegt werden, in denen die Giftwirkung des artfremden Salzes nur durch ein Salz, z. B. NaCl oder $CaCl_2$, gehemmt wurde. Diese Versuche haben nun ein sehr bemerkenswertes Resultat ergeben: Natriumsalze mit einem artfremden Anion, wie Br, J, NO_3, CNS, CH_3COO werden nur durch Chloride entgiftet, resp. an der Giftwirkung verhindert. NaCl, $CaCl_2$ und $MgCl_2$ erwiesen sich als wirkungsvoll, $CaCl_2$ gewöhnlich wirksamer als NaCl. Aber der Unterschied zwischen der Wirksamkeit von NaCl und $CaCl_2$ war klein im Vergleich mit diesem Unterschied bei der Entgiftung von KCl durch diese Salze. Im letzteren Falle war $CaCl_2$ mindestens mehrere hundert Male so wirksam wie NaCl, bei der Entgiftung der artfremden Anionen trat der Unterschied der Kationen viel mehr zurück. Für die Entgiftung von KCl war Na_2SO_4 brauchbar, und zwar war es zweimal so wirksam wie die äquimolekulare Lösung von NaCl, was alles darauf hinweist, daß bei der Entgiftung des giftigen Kations K in erster Linie die Kationen in Betracht kommen. Für die Entgiftung der artfremden Anionen war Na_2SO_4 gänzlich unbrauchbar. Alles weist also darauf hin, daß in diesem Falle die wesentlich entgiftende Wirkung dem Anion Cl zukommt.

Dagegen macht es nicht den Eindruck, als ob für die Entgiftung von Na_2SO_4 Ionen in Betracht kämen, sondern einige Beobachtungen deuten darauf hin, daß hier die Salzmoleküle die entgiftende oder die „gerbende" Wirkung ausüben. Sehr wenig $CaCl_2$ entgiftet eine große Menge Na_2SO_4; $CaSO_4$, $Ca(NO_3)_2$ und $Ca(CH_3COO)_2$ haben aber so gut wie gar keine entgiftende Wirkung. $MgCl_2$ entgiftet Na_2SO_4; $MgSO_4$ hat aber keine entgiftende Wirkung.

Die „gerbende" Wirkung der Salze, insbesondere der Mischung von NaCl + KCl + $CaCl_2$ in dem geeignetsten Verhältnis, dürfen wir uns vielleicht so vorstellen, als ob diese drei Salze mit einem oder mehreren Bestandteilen der Oberfläche der Zellen oder des Tieres (Eiweißkörper?) (molekulare?) Verbindungen eingehen. Diese Verbindungen verleihen der Oberfläche eine besondere Struktur, die eine relativ hohe Undurchgängigkeit garantiert. Kommen die Fische in eine Lösung von etwa $NaNO_3$, so findet nach dem Massenwirkungsgesetze ein Austausch von NaCl und $NaNO_3$ in der Oberflächenlamelle statt, und die $NaNO_3$-Moleküle, die die NaCl-Moleküle aus der Oberflächenlamelle verdrängt haben, können in den Fisch eindringen und die Gehirnsymptome hervorrufen. Fügt man aber der äußeren Lösung NaCl oder $CaCl_2$ in genügender Konzentration zu, so werden diese mit den $NaNO_3$-Molekülen um die Eiweißmoleküle, oder sonstige in Betracht kommende Moleküle, konkurrieren und damit die Zahl der $NaNO_3$-Moleküle verringern, die sonst mit der Oberflächenlamelle in Verbindung treten und damit in das Tier eindringen. Es ist möglich, daß das die Methode ist, durch die Salze in die Zelle eindringen.

Es fehlt nun nicht an direkten Beobachtungen, die darauf hinweisen, daß diese Ansicht begründet ist. P. Hanzlik hat kürzlich die Absorption von Arzneimitteln aus dem Darm untersucht und dabei durch quantitative Analyse des Inhaltes lebender Darmschlingen festgestellt, daß NaJ langsamer aus dem Darm verschwindet, wenn man NaCl zusetzt[1]). Das ist genau dieselbe Erscheinung, die wir bei Fischen beobachteten, nur mit dem Unterschied, daß wir es im letzteren Falle mit der Diffusion des NaJ in den Fisch zu tun haben. NaCl verringert die Geschwindigkeit, mit der NaJ in die Fische eindringt, und darauf beruht wohl die antagonistische Wirkung der Salze, wie wir in diesen Versuchen diskutiert haben.

Zusammenfassung der Ergebnisse.

1. Die giftige Wirkung von $NaNO_3$, NaJ, NaCNS, essigsaurem und buttersaurem Natrium auf Fundulus wird durch Zusatz von NaCl oder $CaCl_2$ gehemmt. Die entgiftende Wirkung von $CaCl_2$ ist etwas größer als die von NaCl.

[1]) Journ. Pharmacol. and Experim. Therap. **3**, 387, 1912.

2. Während jedes dieser Salze durch $CaCl_2$ entgiftet werden kann, hat der Zusatz anderer Calciumsalze keine oder fast keine entgiftende Wirkung. Das weist darauf hin, daß die wesentliche entgiftende Wirkung den Chloriden resp. Chlorionen zukommt; von allen Chloriden kommen aber für diesen Zweck nur NaCl, $CaCl_2$ und in geringerem Grade $MgCl_2$ in Betracht.

3. Na_2SO_4 wird nicht durch NaCl, wohl aber durch kleine Dosen $CaCl_2$ entgiftet. Gleiche Konzentrationen von $CaSO_4$, $CaBr_2$, $Ca(NO_3)_2$ und $Ca(CH_2COO)_2$ hatten keine oder nur eine kaum merkbare hemmende Wirkung. Aus diesen Tatsachen dürfen wir wohl schließen, daß in diesem Falle die entgiftende Wirkung von $CaCl_2$ nicht einem seiner Ionen, sondern dem Molekül zukommt.

4. Eine Mischung von $^m/_8$-NaCl + KCl + $CaCl_2$ in dem Verhältnis, in dem diese Salze im Seewasser enthalten sind, hemmt die Giftwirkung von NaBr, NaJ, $NaNO_3$, NaCNS, $NaCH_3COO$ und $NaCH_3CH_2CH_2COO$ besser als irgendeine andere Lösung.

5. Es wird auf die Möglichkeit hingewiesen, daß die entgiftenden Wirkungen von NaCl, $CaCl_2$ und $MgCl_2$, resp. der Kombination NaCl + KCl + $CaCl_2$ darauf beruhen, daß durch den Einfluß dieser Salze die Oberflächenlamelle der Zellen intakt erhalten und die Diffusion der giftigen Salze in den Fisch verlangsamt wird.

Sonderabdruck aus der Berliner klin. Wochenschr., 1912, Nr. 33.

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Ueber Kriterien der Anaphylaxie.

Bemerkungen zu dem Aufsatz von Friedberger und Moreschi[1]).

Von

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Es darf wohl als gesichert betrachtet werden, dass man bei der Obduktion eines an akuter Serumanaphylaxie verendeten Meerschweinchens beinahe stets die Lungen maximal oder fast maximal gebläht findet. Diese inspiratorische Immobilisation der Lunge wurde zuerst[2]) von Lewis und mir, und später von Biedl und Kraus, näher untersucht, und als die Todesursache für Meerschweinchen bei diesem Typus der Anaphylaxie bewiesen. Ja, wir gingen noch weiter und betrachteten dieses Lungenbild unter gewissen Vorbedingungen, als ein Kriterium der akut tödlichen Meerschweinchenanaphylaxie auch unter solchen Umständen, die das klinische Bild verwischten, wie nach Lähmung des Tieres durch Curare oder durch Rückenmarkszerstörung.

Das Augenfällige dieses Lungenbefundes zog ihm bald eine grosse, aber oft recht kritiklose Beliebtheit zu, und Friedberger und Moreschi ist vollständig beizustimmen, wenn sie schreiben (S. 741): „und wenn man also wohl den Satz aufstellen kann: Kein akuter Anaphylaxietod ohne Lungenblähung, so gilt doch naturgemäss nicht umgekehrt, dass nun der jedesmalige akute Tod eines Meerschweinchens an irgendeiner Vergiftung, bei dem sich das Symptomenbild der Lungenblähung findet, als Anaphylaxie aufzufassen ist". Diese Annahme, dass jede Lungenblähung bei einem akuten Vergiftungstod beim Meerschweinchen notwendigerweise Anaphylaxie bedeute, ist natürlich unzulässig und verwirrungsstiftend, wie ich auch vor kurzem hervorhob[3]).

1) Friedberger und Moreschi, diese Wochenschr., 1912, Nr. 16, S. 741.

2) Der Lungenbefund wurde zuerst von Gay und Southard bemerkt, jedoch wendeten diese Forscher der Tatsache fast gar keine Aufmerksamkeit zu. Siehe Auer und Lewis, Journ. of experim. med., 1910, XII, p. 156—157, p. 160, p. 164, für näheren Aufschluss über den Anteil Gay's und Southard's an dieser Frage.

3) Auer, Journ. of experim. med., 1911, XIV, p. 492—494.

Die Lungenblähung ist nur dann charakteristisch für die akute Meerschweinchenanaphylaxie, wenn sie unter gewissen Versuchsbedingungen erhalten wird. Diese Bedingungen sind die Grundpostulate unseres Begriffes der Anaphylaxie, denn nur durch sie wird das klinische Symptomenbild der Anaphylaxie mit seinen funktionellen Störungen und anatomischen Veränderungen klar und scharf umrissen dargestellt. Diese Grundbedingungen sind wohl bekannt, trotzdem sie in letzter Zeit anscheinend in Vergessenheit gesunken sind: 1. Das zu untersuchende Tier muss durch fremdes Eiweiss sensibilisiert worden sein; 2. die Sensibilisierung bildet sich erst nach einiger Zeit aus (Inkubationsperiode); 3. die zweite oder toxische Injektion desselben Eiweisses, nach Verstrich des Inkubationsstadiums, ruft Symptome und Veränderungen hervor, die nicht bei der ersten Injektion erhalten wurden.

Am klarsten zeigt sich der anaphylaktische Krankheitsprozess, wenn Meerschweinchen mit Pferdeserum präpariert werden. Die erste Injektion, gleichgültig ob subcutan, intraperitoneal oder intravenös, gleichgültig ob $^1/_{100}$ oder 5 ccm, übt zurzeit keinen wahrnehmbaren üblen Effekt aus; das Tier scheint nach einigen Minuten völlig normal zu sein. Wird aber nach drei oder vier Wochen eine Gabe Pferdeserum, die 1 ccm nicht zu überschreiten braucht, intravenös einverleibt, so zeigt das aufgebundene Tier innerhalb weniger Sekunden eine Dyspnöe, die rasch so schwer wird, dass das Zwerchfell und der äussere Luftdruck die Rippenbogen und den Schwertfortsatz des Sternums bei jeder Inspiration einkerben. Nach einer Minute erscheinen lautlose Krämpfe; die Schleimhäute verfärben sich bläulich, und das Blut wird schwärzlich. Die Krämpfe werden jetzt heftiger, nach jeder Inspiration scheint die Brust voller zu sein; die Atmung verlangsamt und vertieft sich; und nach drei bis fünf Minuten sistiert die Atmung vollständig. Das Herz jedoch fährt fort einige Zeit zu schlagen. Die Obduktion zeigt nun die bekannte Lungenblähung mit minimalen Andeutungen von Oedem. Dieses Krankheitsbild und der Lungenbefund sind aber absolut charakteristisch für die akute Anaphylaxie des Meerschweinchens, wenn sie unter den obengenannten Bedingungen erhalten werden: Intravenöse Reinjektion mit derselben Eiweissart, mit der das Tier vorbehandelt wurde. Das charakteristische und von anderen ähnlichen Prozessen differenzierende Moment liegt eben in der Tatsache, dass man den Effekt erst nach der Sensibilisation des Tieres erhält, d. h. nach der zweiten Injekton des zur Sensibilisation gebrauchten fremden Proteins. Dieser Begriff ist von fundamentaler Bedeutung, denn nur er ermöglichte die Erkennung der anaphylaktischen Krankheitsvorgänge als ein Ganzes.

Unter diesen Bedingungen sind all die experimentell ge-

wonnenen Anaphylaxieerscheinungen, das Phänomen von Arthus, die Cutanreaktion (v. Pirquet), die Blutdrucksenkung und Verzögerung der Blutgerinnung beim Hund (Biedl und Kraus), das Lungenphänomen beim Meerschweinchen (Auer und Lewis), der Temperatursturz (Pfeiffer) und der Irritabilitäts- und Kontratilitätsverlust des Herzens beim Kaninchen (Auer), vollständig charakteristisch und als Kriteria brauchbar. Fiedberger und Moreschi kann ich deshalb hier nicht beistimmen, wenn sie bemerken (S. 742), das Krankheitsbild und der Sektionsbefund beim Meerschweinchen sei wenig spezifisch und durch die verschiedensten Substanzen hervorrufbar, und deshalb wäre es nicht gerechtfertigt, von einem spezifischen und „klassischen Symptomenbild" der Anaphylaxie zu sprechen. Man kann das recht wohl tun, aber nur, wenn man die oben hervorgehobenen Grundsätze im Auge behält. Die herrschende Verwirrung ist nur dadurch entstanden, dass man bei Betrachtung von ähnlichen anatomischen Veränderungen die Grundbedingungen der Anaphylaxie aus dem Auge verlor und ohne weiteres auf eine Identität der reaktionsauslösenden Substanzen schloss. So z. B. geben Friedberger's Anaphylatoxin, Eiweissabbauprodukte u. a., wenn intravenös in ein normales Tier eingespritzt, ein Symptomenbild, das jenem der Anaphylaxie stark ähnelt, und deshalb wurde der Krankheitsprozess nach diesen Substanzen als Anaphylaxie gedeutet. Diese Deutung aber ist zurzeit nicht zulässig, denn noch niemand hat den Beweis geliefert, dass die reizauslösenden Substanzen bei der wirklichen Anaphylaxie mit jenen, die die ähnlichen Symptome, z. B. nach Friedberger's Anaphylatoxin, hervorrufen, identisch sind. Aber nur nach Erbringung dieses Beweises darf man von Anaphylaxie nach Anaphylatoxin reden, denn ohne diesen Beweis schliesst man fälschlich wegen der Identität einer biologischen Reaktion auf eine Identität der sie auslösenden Agentia. Dass dieser Schluss ganz und gar falsch ist, ergibt sich aus folgendem Beispiel: Wird der Nerv eines Nervmuskelpräparats mit Glycerin betupft, mit einem heissen Stabe berührt, mit einem Induktionsschlag gereizt, oder wird der Nerv plötzlich gedehnt, so erfolgt in jedem Falle eine Muskelzuckung; darf man aber aus jener Identität der biologischen Reaktion, aus jener Muskelzuckung, schliessen, dass die reizauslösenden Agentia, dass Glycerin, der heisse Stab, der elektrische Reiz, die Nervendehnung, miteinander identisch sind? Alle diese verschiedenen Manipulationen haben wohl dieselbe Reaktion ausgelöst, aber die Gleichheit der Reaktionsantwort bedeutet keineswegs eine Identität der die Reaktion hervorrufenden Eingriffe. Dass solch ein Schluss nicht erlaubt ist, wird wohl jeder zugeben, und doch ist

dieser Schluss genau derselbe, den z. B. Friedberger zieht, wenn er die Symptome nach seinem Anaphylatoxin als anaphylaktische Symptome deutet. Dass die Symptome nach „Anaphylatoxin" jenen der wirklichen Anaphylaxie zum Verwechseln ähnlich sind, beweist bei weitem noch nicht, dass sie durch dasselbe Agens oder dieselben Agentia hervorgebracht worden sind. Bei dem jetzigen Stand unseres Wissens ist es also nicht erlaubt, die Symptome nach erstmaliger Einverleibung von Friedberger's Anaphylatoxin, von Eiweissspaltprodukten u. dergl. als anaphylaktische Symptome zu deuten; zurzeit sind sie nur als anaphylaktoide Symptome anzusprechen, und es muss zukünftiger Arbeit überlassen werden, ob sie wirkliche anaphylaktische Symptome sind oder nicht. Diese Entscheidung kann aber nur dann getroffen werden, nachdem das wirkliche Anaphylatoxin isoliert worden ist, und dieses muss, nach meiner Ansicht, folgende Bedingungen erfüllen: Es muss biologisch aus den Geweben oder Gewebssäften eines Falles wirklicher, aktiver Anaphylaxie isoliert werden; es muss nicht in normalen Tieren vorhanden sein; doch muss es die Symptome, die charakteristisch für die gewählte Tierart sind, auslösen, wenn es in ein normales Tier eingespritzt wird. Sollte sich herausstellen, dass dieselbe Substanz oder dasselbe Substanzgemenge auch z. B. in Friedberger's Anaphylatoxin in genügenden Mengen anwesend ist, dann und nur dann ist Friedberger's Anaphylatoxin ein wirkliches Anaphylatoxin, und die Symptome, die es auslöst, sind anaphylaktische, und nicht anaphylaktoide Erscheinungen.

Bis man das wirkliche Anaphylatoxin in der Hand hat, muss man mit der Diagnose „Anaphylaxie" vorsichtig sein und das Wort nur dann gebrauchen, wenn die oben auseinandergesetzten Grundbedingungen des Begriffs erfüllt sind. Wenn diese Bedingungen als Leitfaden gebraucht werden, so wird man erstens nie im Zweifel sein, ob es sich um anaphylaktische Symptome handelt oder nicht, und zweitens wird das alte Konzept der Anaphylaxie nicht verwischt und nutzlos verflacht, sondern seine Eigenart, die ja seine wissenschaftliche Existenzberechtigung ist, scharf gewahrt. Unter diesen Bedingungen sind all die experimentell ermittelten Tatsachen, die oben teilweise angeführt wurden, streng spezifisch und als Kriteria brauchbar.

Zum Schlusse wäre noch zu bemerken, dass die Meinungsverschiedenheit zwischen Friedberger und mir nur Unterschiede in der Deutung der Phänomena sind; über das Tatsächliche, mit dem dieser unermüdliche und fruchtbare Forscher die Wissenschaft bereichert hat, herrscht wohl kein Zweifel.

Druck von L. Schumacher in Berlin N. 4.

No. 29

Chemische, physikalische und biologische Studien über die aus den drei Monoaminomonocarbonsäuren: Glykokoll, d-Alanin und l-Leucin darstellbaren strukturisomeren Tripeptide.

Von

Emil Abderhalden und Andor Fodor.

Mit 30 Kurvenzeichnungen im Text.

(Aus dem physiologischen Institute der Universität Halle a. S.)

(Der Redaktion zugegangen am 9. August 1912.)

Den folgenden Untersuchungen lagen mehrere Fragestellungen zugrunde. Einmal interessierte es uns, die physikalischen und chemischen Eigenschaften aller strukturisomeren Polypeptide kennen zu lernen, die aus den drei Monoaminomonocarbonsäuren: Glykokoll, d-Alanin und l-Leucin darstellbar sind. Unterscheiden sich die aus diesen drei Aminosäuren aufgebauten Tripeptide in ihren Eigenschaften so scharf, daß sie auf Grund ihres Verhaltens gegenüber bestimmten Lösungs- und Fällungsmitteln usw. voneinander getrennt werden können? Diese Frage war für uns deshalb von so großer Bedeutung, weil der eine von uns (A.) wiederholt beim stufenweisen Abbau von Proteinen verschiedener Art Produkte isolieren konnte, die nach den Resultaten der Elementaranalyse, der totalen Hydrolyse und ihren Eigenschaften ohne Zweifel als Polypeptide angesprochen werden durften, an deren Aufbau eine bestimmte Anzahl bekannter Aminosäuren beteiligt war. Unentschieden blieb die Frage, ob das isolierte Produkt einheitlich war und ferner, in welcher Reihenfolge die einzelnen Aminosäuren sich folgten. Da es in den meisten Fällen unmöglich war, die isolierten Verbindungen zu krystallisieren, so mußte versucht werden, sie durch Fällung zu reinigen. Es gelang in vielen Fällen, auf diesem Wege zu zeigen, daß ein scheinbar ganz einheitliches Produkt doch aus einem Gemisch von strukturisomeren Verbindungen bestand. Die Verfolgung des Drehungsvermögens der einzelnen Fraktionen war in den meisten Fällen wegleitend. Eine Mitteilung der in mehrjähriger Arbeit gewonnenen Resultate erübrigt sich, weil im allgemeinen nur diejenigen Befunde wertvoll sind, denen bestimmt charakterisierte, sicher einheitliche Verbindungen zugrunde liegen.

Zu der großen Schwierigkeit, die durch den Umstand gegeben ist, daß ohne Zweifel die verschiedenartigsten Abbauprodukte von Proteinen ähnliche Eigenschaften aufweisen, kommt noch hinzu, daß es vorläufig fast unmöglich ist, die Gewinnung eines bestimmten Produktes so exakt zu beschreiben, wie es notwendig wäre, um das gleiche Produkt unter den gleichen Bedingungen stets wieder zu erhalten. Die einzelnen Operationen sind so zahlreich und so wechselnd und ferner über einen so großen Zeitraum verteilt, daß es schwer, ja ganz unmöglich ist, den einfachsten Weg zu beschreiben, um die Produkte einer partiellen Hydrolyse zu trennen. Alle nach dieser Richtung unternommenen Untersuchungen stellen tastende Versuche dar. Es fehlt zurzeit eine bestimmte Methodik. Es hängt oft an einer Kleinigkeit, ob eine Krystallisation gelingt oder nicht. Ebenso glückt es oft, durch Fällung zu einheitlichen Verbindungen zu gelangen, während in anderen Fällen immer wieder Gemische ausfallen. Unsere Kenntnisse sind viel zu gering, um zurzeit einzelne Polypeptide auf Grund ihrer Eigenschaften ohne weiteres aus dem bei der Hydrolyse eines Proteins auftretenden Gemische von Spaltprodukten abtrennen zu können. Zeichnet sich auch ein bestimmtes Polypeptid im reinen Zustand durch eine bestimmte Eigenschaft vor allen anderen aus, so wird es doch im allgemeinen nicht gelingen, es auf Grund dieses speziellen Verhaltens zu gewinnen, weil die einzelnen Abbauprodukte sich in ihren Eigenschaften auffallend stark beeinflussen. In Alkohol unlösliche Körper lösen sich, wenn Gemische vorliegen, z. B. spielend leicht in diesem Lösungsmittel. Ja selbst die synthetisch dargestellten Polypeptide zeigen je nach dem Grade der Reinheit ganz verschiedene Eigenschaften. Oft gewinnt man den Eindruck, als würden Isomerien ganz besonderer Art vorliegen, indem ganz reine Polypeptide, die z. B. sich in Alkohol spielend lösten, nach mehrmaligem Eindampfen ihrer Lösung plötzlich in Alkohol vollständig unlöslich werden. Es gibt Polypeptide und vor allem Diketopiperazine, die sich aus keinem Lösungsmittel in Krystallform erhalten lassen, die jedoch beim Sublimieren prachtvolle Krystalle ergeben.

Das Studium einer ganzen Reihe von strukturisomeren Polypeptiden mußte eindeutig ergeben, ob unsere Vermutung, daß im allgemeinen strukturisomere Polypeptide sich durch ihre Löslichkeitsverhältnisse wenig unterscheiden, zutreffend ist. Ferner mußte eine solche Untersuchung positive Anhaltspunkte für diejenigen Eigenschaften der einzelnen Polypeptide ergeben, in denen die Unterschiede groß genug sind, um eine Identifizierung zu ermöglichen. Als eine solche Eigenschaft ergab sich das optische Verhalten. Alle sechs aus den genannten Monoaminosäuren aufgebauten Tripeptide zeigen ein verschiedenes Drehungsvermögen. Würde man in der Natur oder bei der partiellen Hydrolyse eines Proteins oder Peptongemisches eine Verbindung in reinem Zustande abtrennen können, die die Bausteine Glykokoll, d-Alanin und l-Leucin besitzt und ferner das Molekulargewicht eines Tripeptids aufweist, so könnte man ihre Struktur auf Grund ihres spezifischen Drehungsvermögens feststellen. Es dürfte das isolierte Produkt allerdings nicht wesentlich racemisiert sein!

Vergleicht man die in der unten mitgeteilten Tabelle übersichtlich zusammengestellten Eigenschaften der einzelnen Tripeptide, dann erkennt man ohne weiteres, wie schwer es ist, aus dem Hydrolysat von Proteinen einheitliche, wohl charakterisierte Produkte zu gewinnen. Es wird verständlich, weshalb die weitere Forschung auf dem Gebiete der Eiweißchemie so auffallend langsame Fortschritte macht. Ja zurzeit erscheint es fast unmöglich, daß es mit den jetzigen Methoden gelingen könnte, ein Peptongemisch restlos in seine Bestandteile aufzulösen und schließlich ein lückenloses Bild aller Abbaustufen aus einem bestimmten Protein zu geben. Die Frage, ob alle Bausteine im Eiweißmolekül säureamidartig untereinander verknüpft sind, läßt sich einstweilen nur vermutungsweise beantworten. Sind Peptone ausschließlich Gemische von Polypeptiden oder erfolgen auch Spaltungen in noch unbekannter Richtung?

Die Feststellung, daß selbst sechs aus drei Aminosäuren aufgebaute strukturisomere Tripeptide nur schwer voneinander zu unterscheiden sind, zeigt ohne weiteres, wie außerordentlich vorsichtig man bei der

Identifizierung von Proteinen sein muß. Niemals dwir man zwei Eiweißkörper als identisch oder auch nur als ähnlich ansprechen dürfen, weil sie analoge Eigenschaften zeigen. Auch die Resultate der totalen Hydrolyse können hier keine Entscheidung liefern. Alle sechs von uns dargestellten Tripeptide würden bei der totalen Hydrolyse genau die gleichen Bausteine in genau den gleichen Mengenverhältnissen geben. Bedenkt man, daß in den verschiedenen Eiweißarten im allgemeinen alle bisher bekannten Aminosäuren vorkommen und die Menge jeder einzelnen Aminosäure von Protein zu Protein wechselt, dann versteht man, weshalb einzig und allein durch strukturisomere Verschiedenheiten eine gewaltige Zahl verschiedenartiger Eiweißstoffe mit zum Teil ähnlichen, zum Teil aber auch ganz verschiedenen Eigenschaften möglich sind. Jede Pflanzen- und Tierart kann sehr wohl eigenartige Proteine besitzen, ja selbst jeder Zellart kann im gleichen Organismus wiederum Eiweiß besonderer Art zukommen. Unsere physikalischen Methoden sind einstweilen zu grob, um feinere Unterschiede aufzudecken, es sei denn, daß die Kolloidchemie hier Wandlung schaffe. Da jedoch jedes Kolloid und speziell das Eiweiß in den Eigenschaften vom Milieu in hervorragendem Maße abhängig ist, wird man kaum erwarten dürfen, ein klares Bild der Eigenschaften des genuinen Eiweißkörpers an Hand eines isolierten und gereinigten Proteins zu erhalten. Man wird höchstens eine Anzahl verschiedenartiger Proteine unter den gleichen Bedingungen vergleichen können. Schlüsse auf das Verhalten der Eiweißkörper in den einzelnen Zellen wird man jedoch aus diesen Studien kaum ziehen können.

Den Hauptfortschritt auf dem Gebiete der Eiweißchemie erwarten wir immer noch von weiteren Forschungen auf dem Gebiete der partiellen Hydrolyse. Es ist nicht ausgeschlossen, daß neue Methoden entdeckt werden, die rascher zum Ziel führen, als die zurzeit vorhandenen. Eine wesentliche Bereicherung bedeutet schon die Formoltitration nach Soerensen und die Bestimmung des Aminostickstoffs nach van Slyke. Diese Methoden gestatten rasch zu entscheiden, ob man es in einem bestimmten Falle mit einem Polypeptid zu tun hat oder einem Gemisch von freien Amino-

säuren. Ferner kann man leicht feststellen, ob nach erfolgter totaler Hydrolyse der erwartete Aminostickstoffwert vorhanden ist. Beide Methoden haben uns bei der Untersuchung der Spaltprodukte, die bei der partiellen Hydrolyse auftreten, schon ausgezeichnete Dienste erwiesen.

Schließlich wollen wir noch die Frage aufwerfen, ob es möglich wäre, mit den bisherigen Methoden ein künstliches Gemisch der sechs reinen, strukturisomeren Tripeptide in seine Komponenten zu zerlegen. Wir müssen die Möglichkeit einer solchen Trennung als wenig wahrscheinlich bezeichnen. Das Verhalten der Kupfersalze und die verschiedene Fällbarkeit mit Phosphorwolframsäure könnten vielleicht zur Abtrennung des einen oder anderen Tripeptids führen, wenn nicht die große Gefahr bestünde, daß im Gemisch die einzelnen Tripeptide sich in ihren Eigenschaften stark beeinflussen. Würde man nun ein solches Gemisch in der Natur antreffen, dann würde man finden, daß es mit Phosphorwolframsäure fällt. Der Niederschlag löst sich im Überschuß des Fällungsmittels. Das isolierte Produkt würde ein bestimmtes Drehungsvermögen haben. Immer wieder würde man ein Produkt mit gleichen physikalischen Eigenschaften erhalten. Das Molekulargewicht würde immer das gleiche sein. Die Elementaranalyse würde immer die gleichen Werte ergeben. Die totale Hydrolyse würde ferner zu genau den gleichen Aminosäuren führen, endlich würden die Ausbeuten an den einzelnen Bausteinen immer die gleichen sein, und schließlich würde auch z. B. das Verhältnis von N : Cu des dargestellten Kupfersalzes immer annähernd das gleiche bleiben. Der Schluß, daß hier ein einheitlicher Körper vorliegt, wäre gegeben. Kein einziger Eiweißkörper konnte bisher auch nur annähernd durch so viele Befunde charakterisiert werden und doch begegnet man noch häufig der Behauptung, daß die mit durchweg ganz rohen Methoden abgetrennten Proteine einheitlicher Natur seien. Das angeführte Beispiel des Verhaltens von sechs strukturisomeren Tripeptiden, die uns gleichsam als Modelle dienten, dürfte genügen, um zu zeigen, daß wir keinen einzigen der bis jetzt untersuchten Eiweißkörper als einheitlich ansprechen dürfen.

Es ist möglich, daß das eine oder andere der bis jetzt isolierten Proteine einheitlich ist, doch fehlen jegliche Beweise. Die Unsicherheit in der Beurteilung der Natur der zur Untersuchung gestellen Eiweißkörper kann manchen neueren Bestrebungen gegenüber nicht genug betont werden. Es gilt dies sowohl für die rein chemische Forschung, wie für die physikalische. Niemand kann zurzeit den Beweis führen, ob sogenanntes Eiweiß aus einer Verbindung besteht oder nicht vielmehr ein sehr komplexes Gemisch darstellt.

Das Studium des Verhaltens von Gemischen bekannter, wohl definierter Polypeptide wird noch in mancher Hinsicht von großem Interesse werden. Es sei nur einer Beobachtung gedacht, die der eine von uns (A.) gemacht hat. Einzelne Polypeptide, die mit Ammonsulfat aussalzbar sind, verlieren diese Eigenschaft, wenn man bestimmte andere, nicht aussalzbare Polypeptide zufügt. Nun beruhen manche Trennungen von Peptonen auf Aussalzung durch bestimmte Salzlösungen. Eine quantitative Trennung kann auch in solchen Fällen durch die Anwesenheit bestimmter Verbindungen verhindert sein und bei quantitativen Untersuchungen zu ganz unsicheren Schlüssen führen. Wir werden diese Studien fortsetzen.

Wir haben die vorliegende Untersuchung noch aus anderen Gesichtspunkten heraus unternommen. Der eine von uns (A.) hat wiederholt darauf hingewiesen, daß die optisch aktiven Polypeptide ein ganz vorzügliches Substrat zu qualitativen und quantitativen Fermentstudien abgeben.[1]) Es läßt sich im allgemeinen ganz scharf feststellen, an welcher Stelle Polypeptide zuerst angegriffen werden. Da das Drehungsvermögen aller Bruchstücke eines optisch-aktiven Polypeptids genau bekannt ist, läßt sich aus der eintretenden Drehungsänderung des Substrat-Ferment-Gemisches genau ableiten, in was für Teile das angewandte Polypeptid zerfällt. Man kann die erhobenen Resultate dazu verwenden, um Produkte, die man unter den Abbauprodukten von Proteinen ge-

[1]) Vgl. zu den hier erörterten Fragestellungen: Emil Abderhalden: Schutzfermente des tierischen Organismus. Ein Beitrag zur Kenntnis der Abwehrmaßregeln des tierischen Organismus gegen körper-, blut- und zellfremde Stoffe. J. Springer, Berlin, 1912.

funden hat, zu identifizieren. Die Art des Abbaus durch ein bestimmtes Ferment könnte als weiterer Beweis für das Vorliegen eines bestimmten Polypeptids dienen. Außerdem kann man mit Hilfe der optischen Methode den Zeitpunkt genau festlegen, in dem ein bestimmter Abbau sein Maximum erreicht hat. Man kann dann die Fermentwirkung unterbrechen und die zu erwartenden Bruchstücke einzeln abscheiden und identifizieren.

Wir haben zunächst den Abbau der dargestellten Tripeptide mit Hilfe von Hefemazerationssaft studiert und gefunden, daß in allen Fällen der Abbau in ganz typischer Weise einsetzte. Stets wurde die Aminosäure abgespalten, die die Aminogruppe trug. In einigen Fällen war die Entscheidung der Art der Spaltung dadurch sehr erschwert, daß die möglichen Spaltprodukte sich im Drehungsvermögen nicht scharf genug unterschieden. Diese Fälle sind in der unten mitgeteilten Tabelle durch ein ? kenntlich gemacht. Hier müßte die Isolierung der Spaltprodukte Klarheit schaffen, doch reichte das uns zur Verfügung stehende Material in keinem Falle aus.

Preßsaft aus Leber zeigte eine analoge Spaltung, wie der Hefemazerationssaft, dagegen spaltete aktivierter Pankreassaft d-Alanyl-glycyl-l-leucin, l-Leucyl-d-alanyl-glycin und l-Leucyl-glycyl-d-alanin in anderer Weise. Wir dachten daran, daß die Konzentration des Substrates oder des Fermentes oder auch die Reaktion des Gemisches einen Einfluß auf die Art des Abbaus haben könnte. Wir haben deshalb besondere Versuche angestellt, um diese Möglichkeiten zu ergründen. Wir erhielten in allen Fällen immer die gleiche Art des Abbaus.

Preßsaft von einer normalen menschlichen Pankreasdrüse ergab die gleiche Spaltung, wie der Leberpreßsaft. Ebenso verhielt sich Preßsaft aus der Pankreasdrüse eines Falles von Diabète broncé, eines gewöhnlichen Diabetes melitus[1]) und einer Basedowschen Krankheit. Bemerkenswert ist die rasche Spaltung der angewandten Polypeptide durch den Pankreaspreßsaft bei Diabetes.

Aus diesen Beobachtungen scheint hervorzugehen, daß die Zellfermente die Tripeptide stets in der gleichen Art ab-

[1]) Dieser Fall ist nur qualitativ geprüft worden.

bauen. Es entstehen somit in diesen Fällen immer die gleichen Abbaustufen. Ein anderes Verhalten zeigt nur der aktivierte Pankreassaft. Er spaltet in anderer Richtung. Dieses Verhalten ist geeignet, um in zweifelhaften Fällen Pankreassaft scharf von den sogenannten intracellulären Fermenten zu trennen. Es wird von großem Interesse sein, festzustellen, ob bei anderen Polypeptiden analoge Unterschiede gegenüber den Zellfermenten sich ergeben.

Im Anschluß an eine Beobachtung von Euler[1]) haben wir geprüft, ob Phosphate den Abbau durch bestimmte Fermentlösungen beschleunigen. Wir fanden beim Hefemazerationssaft keinen Einfluß, dagegen war eine sehr beträchtliche Beschleunigung wahrnehmbar, als wir Pankreas- und Leberpreßsaft verwendeten. Diese Beobachtungen müssen erweitert werden, bevor bestimmte Schlüsse und neue Fragestellungen an sie geknüpft werden können.

Schließlich haben wir die Darstellung von stereoisomeren Polypeptiden noch unternommen, um Substrate zu erhalten, die es ermöglichen, bestimmte Lebewesen, vor allem Mikroorganismen zu unterscheiden und auf ihre Eigenart zu prüfen. Es wäre wohl denkbar, daß ein bestimmtes Lebewesen ein bestimmtes Tripeptid abbaut und die anderen strukturisomeren Tripeptide unberührt läßt. Ferner wäre es denkbar, daß zwei verschiedene Bakterien ein bestimmtes Tripeptid zwar gemeinsam abbauen, jedoch den Abbau in verschiedener, ganz typischer Weise durchführen. Wir halten derartige Untersuchungen für außerordentlich wichtig. Sie würden ohne Zweifel mancherlei Einblicke in die Eigenart des Stoffwechsels der einzelnen Mikroorganismen eröffnen und uns in den Stand setzen, manche dieser Lebewesen direkt zu erkennen. Wir haben vorläufig Bact. coli und den Typhusbacillus untersucht. Auffallenderweise wurde kein einziges der untersuchten fünf Tripeptide (nicht geprüft wurde l-Leucyl-glycyl-d-alanin) abgebaut. Als Nährlösung verwandten wir die Fränkensche Lösung. Als

[1]) Vgl. hierzu: Hans Euler, Verhalten der Kohlenhydratphosphorsäureester im Tierkörper. Diese Zeitschrift, Bd. 79, S. 375 (395), 1912.

einzige Stickstoffquelle wurden die Tripeptide zugesetzt. Versuche, die etwa 5 Jahre zurückliegen, hatten bereits gezeigt, daß viele Mikroorganismen Polypeptide nur schwer angreifen. Es wäre sehr verlockend, auf breiterer Grundlage die angeregten Fragestellungen zu verfolgen. Leider fehlen uns die Mittel, um derartige Untersuchungen mit Erfolg durchzuführen.

Die vorliegenden Untersuchungen waren nur möglich, weil der Vorstand des Rockefeller Institute for medical Research dem einen von uns (A.) in liebenswürdigster Weise Mittel zur Verfügung stellte. Es ist uns eine angenehme Pflicht, auch an dieser Stelle unseren herzlichsten Dank abzustatten.

Die folgende Tabelle gibt die hauptsächlichsten Resultate der ganzen Untersuchung wieder. Sie enthält die physikalischen und chemischen Eigenschaften der dargestellten Verbindungen und ferner die Art der Spaltung durch die einzelnen Fermentlösungen. Es sei noch hinzugegfügt, daß alle Tripeptide mit Ausnahme von l-Leucyl-d-alanyl-glycin (l-Leucyl-glycyl-d-alanin haben wir nicht geprüft) mit Phosphorwolframsäure fallen. Die Fällung löst sich leicht im Überschuß des Fällungsmittels. Einzig der Niederschlag mit d-Alanyl-glycyl-l-leucin löst sich etwas schwerer.

Eine weitere Tabelle zeigt in übersichtlicher Weise, in welcher Weise die Tripeptide von den einzelnen Fermentlösungen abgebaut wurden. Sie zeigt, wie die Art des Abbaus aus der Änderung des Drehungsvermögens erschlossen wurde.

Endlich sei noch erwähnt, daß wir uns mit der Frage nach der Aktivierung von Fermenten beschäftigt haben. Es wird allgemein angenommen, daß das Trypsinzymogen durch die Enterokinase des Darmsaftes aktiviert wird. Es schien uns nicht ausgeschlossen, daß manche Aktivatoren in der Art wirken, daß sie das Substrat als solches so verändern, daß es durch das sog. Fermentzymogen nunmehr abgebaut werden kann. Man müßte in diesem Falle von einer Aktivierung des Substrates sprechen und den Begriff der Fermentvorstufe fallen lassen. Bis jetzt konnten wir (vgl. Versuche S. 37) eine primäre Veränderung eines Substrates durch die Enterokinase nicht feststellen. Immerhin erscheint es uns von

Tabelle I.

	F. P. (unkorr.)	Löslichkeitsverhältnisse	Spezifische Drehung $[\alpha]_D^{20} =$	Literatur	
Chloracetyl-d-alanyl-l-leucin	169°	Wasser: k. sl., h. s. l. Alkohol: s. l. Äther: z. s. l. Essigäther: k. sl., h. l. Petroläther: u.	— 51,58° in Alkohol	—	
Chloracetyl-l-leucyl-d-alanin	136—7°	Wasser: k. z. l., h. s. l. Alkohol: s. l. Äther: k. sl., h. z. sl. Essigäther: k. z. s., h. l. Petroläther: u.	— 41,52° in Alkohol	—	
α-d-Brompropionyl-glycyl-l-leucin	152°	Wasser: k. z. l. Alkohol: s. l. Äther: k. sl., h. z. l. Essigäther: k. sl., h. l. Petroläther: u.	+ 14,71° in Alkohol	—	Abkürzungen: k. = kalt h. = heiß s. = sehr z. = ziemlich sl. = schwer löslich l. = leicht löslich u. = unlöslich
α-d-Brompropionyl-l-leucyl-glycin	154—5°	Wasser: k. sl., h. s. Alkohol: s. l. Äther: k. und h. sl. Essigäther: k. sl., h. l. Petroläther: u.	— 24,84° in Alkohol	—	
α-d-Bromisocapronyl-d-alanyl-glycin	129°	Wasser: k. sl., h. l. Alkohol: s. l. Äther: k. sl., h. l. Essigäther: k. z. sl., h. l. Petroläther: u.	— 2,52° in Alkohol	—	
α-d-Bromisocapronyl-glycyl-d-alanin.	188° (korr.)	Wasser: k. sl., h. l. Alkohol: } nicht Äther: } bestimmt Essigäther: } Petroläther: u.	+ 20,4° in Alkohol	E. Fischer und J. Steingröver, Annalen der Chemie und Pharmacie, Bd. 365, S. 167 (1909)	

Tabelle I. — Fortsetzung.

Tripeptid	Zersetzungspunkt (unkorr.)	Löslichkeitsverhältnisse	Spezifische Drehung $[\alpha]_D^{20} =$	Atomverhältnis Cu : N des amorphen Cu-Salzes	Biuretreaktion	Fermentative Spaltung erfolgt bei Anwendung von Hefemazerationssaft in	bei Anwendung von Leberpreßsaft in	bei Anw. von mit Darmsaft aktiviert. Pankreaspräp. in	bei Anw. v. menschlichem Pankreaspreßsaft in	bei Anw. von normalem Pankreaspreßsaft und von solchem bei Diabetes und Morbus Basedowii
Glycyl-d-alanyl-l-leucin	239—40°	H_2O: k. z. l., h. s. l. C_2H_5OH: u. (absol.)	— 89,86° in H_2O	1 Cu : 4 N	stark	Glykokoll + ? d-Alanyl-l-leucin	?	?	?	?
Glycyl-l-leucyl-d-alanin	235—36°	H_2O: k. s. l. C_2H_5OH: u.	— 59,04° in H_2O	1 Cu : 4 N	stark	Glykokoll + ? l-Leucyl-d-alanin	?	?	?	?
d-Alanyl-glycyl-l-leucin	243°	H_2O: k. s. l. C_2H_5OH: u.	— 11,20° in H_2O	1 Cu : 4 N	stark	d-Alanin + Glycyl-l-leucin	d-Alanin + Glycyl-l-leucin	d-Alanyl-glycin + l-Leucin	d-Alanin + Glycyl-l-leucin	d-Alanin + Glycyl-l-leucin (Diabetes)
d-Alanyl-l-leucyl-glycin	246—47°	H_2O: k. s. l. C_2H_5OH: u.	— 30,41° in H_2O	1 Cu : 4 N	stark	d-Alanin + l-Leucyl-glycin	d-Alanin + l-Leucyl-glycin	d-Alanin + l-Leucyl-glycin	d-Alanin + l-Leuyl-glycin	d-Alanin + l-Leucyl-glycin (Diabet. u. Basedow)
l-Leucyl-d-alanyl-glycin	252—53°	H_2O: k. sl., h. z. l. C_2H_5OH: u.	— 17,31° in H_2O	1 Cu : 3 N	schwach	l-Leucin + d-Alanyl-glycin	l-Leucin + d-Alanyl-glycin	l-Leucyl-d-alanin + ? Glykokoll	l-Leucin + d-Alanyl-glycin	l-Leucin + d-Alanyl-glycin (Basedow)
l-Leucyl-glycyl-d-alanin [1])	249°	H_2O: l. löslich C_2H_5OH: u.	+ 20,3° in H_2O	Nicht festgestellt	violettblaue Färbung	l-Leucin + Glycyl-d-alanin [2])	—	l-Leucyl-glycin + d-alanin [2])	—	—

norm. menschl. Pankreaspreßsaft spalten in gleicher Weise

[1]) E. Fischer und J. Steingröver, loc. cit.

[2]) Emil Abderhalden und A. H. Kölker, Weiterer Beitrag zur Kenntnis des Verlaufs der fermentativen Polypeptidspaltung unter verschiedenen Bedingungen, Diese Zeitschrift, Bd. 54, S. 363 (1908), und Bd. 55, S. 416 (1908).

Tabelle II.

I. — 89,86°
Glycyl-d-alanyl-l-leucin
— 50° —10,34°

— 89,86°
Glycyl-d-alanyl-l-leucin
0° — 17,21°

II. — 59,04°
Glycyl-l-leucyl-d-alanin
— 35,09° + 2,7°

— 59,04°
Glycyl-l-leucyl-d-alanin
0° + 10°

Bei diesen beiden Tripeptiden ist der Verlauf der Spaltung, sichtbar gemacht im optischen Rohr, nicht scharf erkennbar, da die Spaltungsprodukte, ebenso wie die Tripeptide selbst, linksdrehend oder nur wenig rechtsdrehend sind. Die Spaltprodukte (Dipeptide) unterliegen bereits der weiteren Spaltung, bevor noch die völlige Spaltung des Tripeptids zu denselben erreicht ist, wodurch der Verlauf der Spaltung verdeckt wird.

III. — 11,20°
d-Alanyl-glycyl-l-leucin
+ 50,2° —10,34° — aktiviert. Pankreaspräparat.

— 11,20°
d-Alanyl-glycyl-l-leucin
+ 2,7° — 35,09° — Hefesaft, Leber-Pankreaspreßsäfte.

IV. — 30,41°
d-Alanyl-l-leucyl-glycin
— 17,21° 0° — nicht gefunden.

— 30,41°
d-Alanyl-l-leucyl-glycin
+ 2,7° + 85,99° — Hefesaft, Leber-Pankreaspreßsäfte, aktiv. Pankreaspräp.

V. — 17,31°
l-Leucyl-d-alanyl-glycin
+ 10° 0° — aktiviert. Pankreaspräparat.

— 17,31°
l-Leucyl-d-alanyl-glycin
—10,34° + 50,2° — Hefesaft, Leber-Pankreaspreßsäfte.

VI. + 20,3°
l-Leucyl-glycyl-d-alanin
+ 85,99° + 2,7° — aktiviert. Pankreaspräparat.

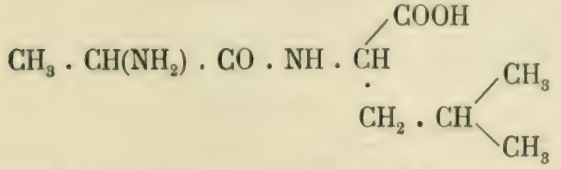
+ 20,3°
l-Leucyl-glycyl-d-alanin
—10,34° — 50° — Hefepreßsaft.

Wichtigkeit, daß in jedem einzelnen Falle die Bedeutung und die Wirkungsart der sog. Aktivatoren und vor allem ihr Angriffspunkt genau studiert wird.

Experimenteller Teil.

Glycyl-d-alanyl-l-leucin.

d-Alanyl-l-leucin.[1])



10 g l-Leucin (1 Mol.) wurden in 76,3 ccm n-NaOH (1 Mol.) gelöst und unter starker Kühlung mit 15,9 g d-Brompropionylchlorid (1,2 Mol.) und portionenweiser Zufügung von 114,4 ccm n-NaOH (1,5 Mol.) gekuppelt. Das nach dem Ansäuern ausgefallene Öl wurde wiederholt in Äther gelöst und mit Petroläther ausgefällt und mit letzterem verrieben. Es erstarrte schließlich bei starker Kühlung mittels Kältegemisches zu einer harten krystallinischen Masse, die jedoch bei gewöhnlicher Temperatur wieder in den sirupösen Zustand überging. Ebenso verhielt sich jener Anteil des öligen Kupplungsproduktes, der aus dem ätherischen Extrakte des Kochsalzrückstandes gewonnen wurde. Letzterer hinterblieb beim Eindampfen der schwachsalzsauren Flüssigkeit unter stark vermindertem Druck.

Der zähe Sirup (11 g) wurde mit der zehnfachen Menge 25-%igen wässerigen Ammoniaks bei Zimmertemperatur 5 Tage lang amidiert, die Lösung sodann im Vakuum eingedampft und der verbleibende Rückstand mit Wasser aufgenommen. Diese Lösung wurde mit Silbersulfat so lange geschüttelt, bis das gesamte Brom ausgefällt war. Das Filtrat von Bromsilber wurde nach dem Fällen des Silbers mit Schwefelwasserstoff und Vertreiben des Überschusses an diesem Gas durch einen Luftstrom mit Barytlösung quantitativ von der Schwefelsäure befreit. Das Filtrat vom Baryumsulfat wurde nach starkem Eindunsten bei 12 mm Druck mit soviel absolutem Alkohol versetzt, bis

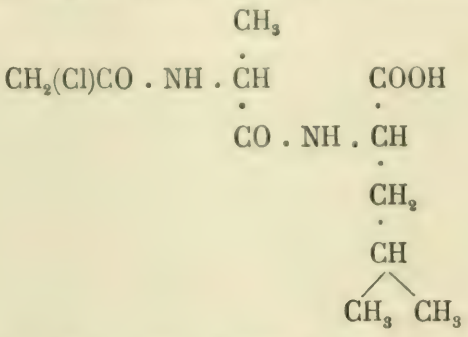
[1]) E. Fischer, Synthese von Polypeptiden, XVII. Ber. d. Deutsch. Chem. Gesellsch., Jg. 40, S. 1754, 1907.

eine auch beim Kochen bleibende Trübung verblieb. Beim langsamen Abkühlen schied sich ein weißer krystallinischer Brei ab. Er wurde abgesaugt und gewaschen (5 g).

Bestimmung des Drehungsvermögens. 0,1160 g Substanz in H_2O gelöst. Gesamtgewicht der Lösung: 6,5872 g. d = 1,004. α (im ½-dm-Rohr) = −0,15°. $[\alpha]_D^{20°} = -16{,}96°$.

Die Mutterlauge enthielt noch ca. 1 g des Dipeptids.

Chloracetyl-d-alanyl-l-leucin.



5 g des Dipeptids obiger Drehung wurden in 25 ccm n-Natronlauge (1 Mol.) gelöst und mit 3,5 g Chloracetylchlorid (1⅓ Mol.) und 37,1 ccm n-Natronlauge (1,5 Mol.) in der gewohnten Weise gekuppelt. Die filtrierte Lösung gab beim schwachen Übersäuern sofort eine farblose krystallinische Ausscheidung, die abgetrennt und ausgewaschen wurde. Ausbeute: 5 g. Das so erhaltene Produkt schmilzt bei 169° (unkorr.) zu einer klaren Flüssigkeit. Nach dem Umkrystallisieren aus siedendem Essigester und Auswaschen der beim Verdunsten erhaltenen Krystalle mit Petroläther steigt der Schmelzpunkt auf 175° (unkorr.). Das beim Ansäuern direkt ausfallende Produkt besteht aus mikroskopischen Blättchen. Eine aus heißem Alkohol umkrystallisierte Probe gibt jedoch schon makroskopisch sichtbare, vogelfederartig gruppierte und gekrümmte Nadeln. Beim Eindunsten der salzsauren Mutterlauge unter vermindertem Drucke (12 mm) schieden sich noch 0,5 g der gleichen Krystalle aus. Der feste Eindampfrückstand hingegen gab nach dem Ausziehen mit Essigester, Trocknen des Auszuges mit Magnesiumsulfat und Verdunsten des Lösungsmittels einen öligen Rückstand, der nicht zur Krystallisation gebracht werden konnte.

Löslichkeitsverhältnisse:

Äther : auch in der Wärme ziemlich schwer löslich.
Essigäther : kalt schwer, heiß leicht löslich.
Petroläther: unlöslich.
Alkohol : sehr leicht löslich.
Wasser : kalt schwer, heiß sehr leicht löslich.

Analysen:

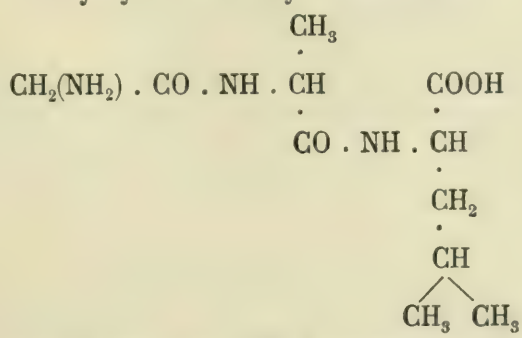
0,1096 g Substanz: 0,0542 g AgCl
0,1221 g » : 0,2142 g CO_2
0,0751 g H_2O
0,1211 g » : 8,90 ccm $^1/_{10}$-n-H_2SO_4 (Kjeldahl).

Berechnet für $C_{11}H_{19}N_2ClO_4$ (= 278,61):		Gefunden:
C	47,38%	47,84
H	6,87%	6,88
Cl	12,73%	12,23
N	10,06%	10,30

Optisches Verhalten:

0,4166 g Substanz in absolutem Alkohol gelöst. Gesamtgewicht = 6,4154 g. d = 0,806; α (5 cm-Rohr) = — 1,35°. $[\alpha]_D^{20°}$ = — 51,58°.

Glycyl-d-alanyl-l-leucin:



4,5 g vom obigen bei 175° schmelzenden Chlorkörper wurden 6 Tage lang mit der 5 fachen Menge 25%igen Ammoniaks bei Zimmertemperatur amidiert. Nach dem Verdunsten der Lösung im Vakuum wurde mit ganz wenig Wasser wieder aufgenommen und die Lösung am Wasserbade mit absolutem Alkohol bis zur bleibenden Trübung versetzt. Beim Abkühlen im Eisschrank schied sich das Tripeptid in dicken, schnee-

weißen Flecken aus, die abgesaugt und ausgewaschen wurden (2,9 g). Das so gewonnene Produkt erwies sich als ein halogenfreier, geschmackloser, bei 239—40° (unkorr.) unter Aufschäumen und Braunfärbung schmelzender Körper (239° beginnendes Sintern und Bräunung). Derselbe löst sich bereits in kaltem Wasser ziemlich leicht auf, viel leichter jedoch in der Wärme. In absolutem Alkohol ist das Tripeptid vollständig unlöslich. Die wässerige Lösung reagiert gegen Lackmus schwachsauer. Biuretreaktion sehr stark.

Die Reinigung wurde durch Umkrystallisation aus Wasser versucht. Beim Verdunsten der wässerigen Lösung erhält man makroskopische, feine, farblose Nadeln als Ausscheidung, die konstant bei 239—40° unter Zersetzung schmelzen. Die Mutterlauge gibt beim vollständigen Verdunsten die gleichen Nadeln vom gleichen Schmelzpunkt.

Analysen:

0,1221 g Substanz: 0,2307 g CO_2
0,0874 g H_2O
0,0986 g » : 11,23 ccm $^n/_{10}$-H_2SO_4 (Kjeldahl).

Berechnet für $C_{11}H_{21}O_4N_3$ (= 259,19):	Gefunden:
C 50,93	51,53
H 8,16	8,01
N 16,21	15,95

Optisches Verhalten:

0,2657 g Substanz in Wasser gelöst. Gesamtgewicht der Lösung = 7,8205 g. d = 1,009; α (im 5-cm-Rohr) = — 1,54°. $[\alpha]_D^{20}$ = — 89,85°.

Kupferverbindung:

Beim längeren Kochen der wässerigen Tripeptidlösung mit überschüssigem Kupferoxyd erhält man nach dem Filtrieren eine blauviolette Lösung. Dieselbe hinterläßt beim Verdunsten eine hellblaue, amorphe, glasige Masse. Dieselbe ist in absolutem Alkohol ziemlich schwer löslich, leicht in verdünntem.

Analysen:

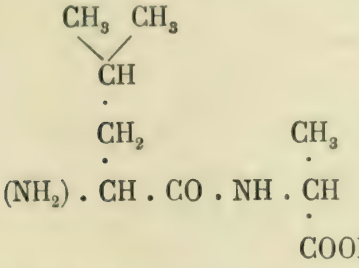
0,1382 g Substanz: 0,0241 CuO
0,1286 g » : 11,30 ccm $^n/_{10}$-H_2SO_4 (Kjeldahl).

Hieraus ergibt sich ein Atomverhältnis von 1 Cu : 4 N.
Für 4 $C_{11}H_{21}O_4N_3 \cdot 3\ CuO \cdot 6\ H_2O$ (= 1381,68).

Berechnet:	Gefunden:
13,81% Cu	13,93% Cu
12,17% N	12,31% N.

Glycyl-l-leucyl-d-alanin.

l-Leucyl-d-alanin.[1])



Zur Darstellung des d-Bromisocapronyl-d-alanins wurden 1,25 Mol. Alanin in 1,25 Mol. n-Natronlauge gelöst und mit 1 Mol. d-Bromisocapronylchlorid + 1,3 Mol. n-Natronlauge wie gewöhnlich gekuppelt. Beim Übersäuern gelangte sogleich ein öliges Produkt zur Ausscheidung, das mit Äther aufgenommen wurde. Die eingeengte, vorher getrocknete ätherische Lösung wurde nun mit Petroläther versetzt und der ausgefallene trübe Sirup mit letzterem längere Zeit und wiederholt gewaschen.

Nach nochmaligem Umfällen mit Petroläther aus der essigätherischen Lösung erstarrte das zunächst wieder nur als zähes Öl ausfallende Produkt im evakuierten Exsikkator zu einer festen Krystallmasse. Sie wurde mit größeren Mengen Petroläthers gründlich gewaschen, abgesaugt und die Umfällung aus Essigäther wiederholt. Diesmal wurde jedoch durch eine ungenügende Hinzufügung von Petroläther die plötzliche Ausfällung vermieden und die Lösung der langsamen Verdunstung ausgesetzt. Hierbei gelangten schöne Nadeln vom d-Bromisocapronyl-d-Alanin zur Auskrystallisation, die abgesaugt, gewaschen und im evakuierten Exsikkator über Schwefelsäure getrocknet wurden.

[1]) E. Fischer, Synthese von Polypeptiden, XV. Ber. d. Deutsch. Chem. Gesellsch., Jg. 39, S. 2893, 1906.

Optische Bestimmung:

0,2525 g Substanz in absolutem Alkohol gelöst; Gesamtgewicht der Lösung 0,2380 g. d = 0,802. α (im 5-cm-Rohr) = + 0,68°. $[\alpha]_D^{20}$ = + 20,95°.

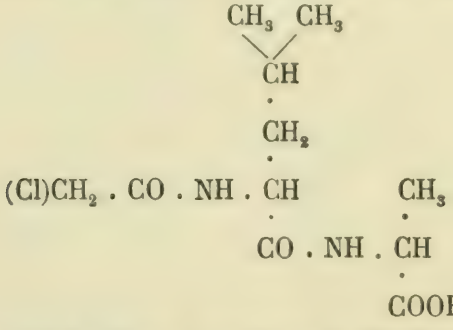
Die so erhaltenen Krystalle von d-Bromisocapronyl-d-alanin wurden mit der 5fachen Menge 25%igen Ammoniaks 5 Tage lang bei Zimmertemperatur amidiert und hernach, wie oben bei der Darstellung von d-Alanyl-l-leucin erwähnt, weiter verfahren. Das Filtrat vom Baryumsulfat wurde auch hier bei 12 mm eingedunstet und die stark konzentrierte Lösung mit überschüssigem absoluten Alkohol versetzt. Die im Eisschrank allmählich entstandene gelatinös erscheinende Ausscheidung wurde abgesaugt und mit absolutem Alkohol gewaschen (F. 240°). Durch Verdunstung der Mutterlauge wurden noch weitere 2 Fraktionen gewonnen, eine vom Schmelzpunkt 240°, die zweite zeigte F. = 235°.

Die bei 240° schmelzenden Fraktionen wurden in siedendem Methylalkohol aufgelöst. Beim langsamen Verdunsten erhält man schöne, schneeweiße feine Nadeln von F. 254—55° (unkorr.), die von der Mutterlauge abgesaugt und mit kaltem Methylalkohol gewaschen wurden.

Optische Untersuchung:

0,1934 g Substanz in Methylalkohol gelöst; Gesamtgewicht der Lösung 5,0505 g. d = 0,790. α (im 5-cm-Rohr) = + 0,30°. $[\alpha]_D^{20}$ = + 19,84°.

Chloracetyl-l-leucyl-d-alanin.



6,3 g l-Leucyl-d-alanin wurden in 32,3 ccm n-Natronlauge (1 Mol.) gelöst und in der gewohnten Weise mit 4,4 g Chlor-

acetylchlorid (1,25 Mol.) + 46,4 ccm n-Natronlauge (1,5 Mol.) gekuppelt. Beim Übersäuern entstand eine sirupöse Ausscheidung, von welcher die Lösung abdekantiert wurde. Letztere wurde hierauf bei 12 mm Druck vollständig eingedampft und der Trockenrückstand mit heißem Essigäther erschöpft. Der mit Magnesiumsulfat getrocknete Auszug gab beim Verdunsten ebenfalls einen sirupösen Rückstand.

Die beiden Anteile des sirupösen Kupplungsproduktes wurden hierauf getrennt und Äther mehrmals verrieben, wobei das Produkt krystallinisch wurde und abgesaugt werden konnte. Beide Anteile zeigten nach dem Auswaschen mit Äther und Trocknen F. = 132° (124° Sintern). Die Gesamtausbeute betrug 4,5 g. Behufs Reinigung wurde das Produkt aus heißem Essigäther umkrystallisiert. Beim langsamen Verdunsten schieden sich weiße Krystalle aus, die abgesaugt und mit Petroläther gewaschen wurden. F. 136—137°. Beim Schmelzen entsteht eine klare Flüssigkeit. Bei ungefähr 130° tritt Sintern ein.

Die aus der alkoholischen Lösung durch Verdunstung gewonnenen Krystalle zeigen unter dem Mikroskope unscharfkantige Prismen.

Die ätherischen Waschflüssigkeiten hinterließen beim Eindunsten eine sirupöse Masse, die beim Verreiben mit Äther und Chloroform in ein halbfestes Produkt umgewandelt wurde. Dasselbe erstarrte zum Teil beim Verreiben mit Petroläther. Durch Umfällen dieser Masse aus ihrer essigätherischen Lösung mit Petroläther wurden weitere 1,2 g bei 136° schmelzender Krystalle gewonnen, die jedoch schon bei 100° zu sintern begannen.

Analysen.

Zur Analyse wurde die bei 136—137° schmelzende Partie verwendet.

0,0951 g Substanz: 0,1652 g CO_2; 0,0587 g H_2O.
0,1138 g » 0,0570 g AgCl.
0,1041 g » 7,62 ccm $^1/_{10}$-n-H_2SO_4 (Kjeldahl).

Berechnet für $C_{11}H_{19}N_2ClO_4$ (278,61):		Gefunden:
C	47,38%	47,38%
H	6,87%	6,91%
Cl	12,73%	12,38%
N	10,06%	10,25%.

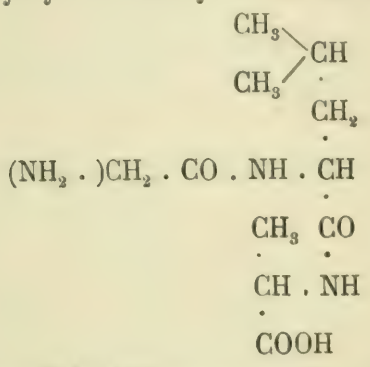
Optisches Verhalten:

0,2667 g Substanz in absolutem Alkohol gelöst; Gesamtgewicht der Lösung 5,5569 g, d = 0,803; α (im 5-cm-Rohr) = — 0,80°. $[\alpha]_D^{20} = -41{,}52°$.

Löslichkeitsverhältnisse:

Äther: kalt sehr schwer lösl., heiß zieml. schwer lösl.,
Essigäther: » zieml. schwer lösl., » leicht löslich,
Petroläther: unlöslich,
Alkohol: sehr leicht löslich,
Wasser: kalt ziemlich löslich, heiß sehr leicht löslich.

Glycyl-l-leucyl-d-alanin.



4 g des obigen Chlorkörpers wurden mit der fünffachen Menge 25%igen wässerigen Ammoniaks 5 Tage hindurch bei Zimmertemperatur amidiert. Die weitere Verarbeitung war die gleiche, wie beim vorherigen Tripeptid. Es wurden 2,5 g eines aus der wässerigen Lösung und Alkohol in großen weißen Flocken ausgefallenen Produktes gewonnen, das F. = 235 bis 236° unter dunkelbrauner Färbung, bald mit, bald ohne Gasentwicklung aufweist. Bei 221° tritt leichte Bräunung ein. Das Tripeptid ist bereits in kaltem Wasser spielend leicht löslich, unlöslich in absolutem Alkohol. Es zeigt sehr deutliche Biuretreaktion.

Zwecks Reinigung wurde das Produkt in wenig Wasser gelöst und die Lösung am Wasserbade eingedunstet. Das

Tripeptid scheidet sich hierbei auf der Oberfläche in konzentrisch angeordneten, seidenglänzenden Nadeln aus, die so dicht aneinandergelagert sind, daß sie leicht dentritische Blättchen vortäuschen. Sowohl diese als auch der beim völligen Eindunsten der Mutterlauge hinterbleibende Rückstand zeigen F. = 235—36°.

Analysen.

0,1591 g Substanz: 0,2950 g CO_2, 0,1158 g H_2O.
0,0979 g » 11,12 ccm $^1/_{10}$-n-H_2SO_4.

Berechnet für $C_{11}H_{21}O_4N_3$ (259,19):		Gefunden:
C	50,93%	50,57%
H	8,16%	8,14%
N	16,21%	15,91%.

Optisches Verhalten:

0,2415 g Substanz in Wasser gelöst. Gesamtgewicht der Lösung 9,8254 d = 1,006; α (in 1-dm-Rohr) = — 1,46°.

$$[\alpha]_D^{20} = -59{,}04°.$$

Kupferverbindung.

Die Darstellung geschah genau so, wie es beim Glycyl-d-alanyl-l-leucin beschrieben wurde. Die wässerige Lösung der Kupferverbindung ist rotviolett gefärbt. In absolutem Alkohol ist das Produkt ziemlich löslich, leichter in verdünntem Alkohol.

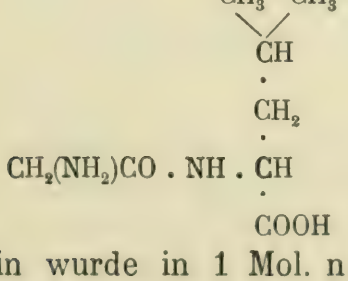
Analyse:

0,1395 g Substanz: 0,0244 g CuO = 13,97% Cu.

Vgl. den entsprechenden Wert bei der Kupferverbindung des Glycyl-d-alanyl-l-leucins.

d-Alanyl-glycyl-l-leucin.

Glycyl-l-leucin.[1])



1 Mol. l-Leucin wurde in 1 Mol. n-Natronlauge gelöst

[1]) Jos. Steingroever, Synthese einiger Polypeptide mit Beziehung zu dem Isobutyldiketopiperazin. Diss., Berlin (Chem. Inst. d. Univ.), 1907.

und wie vorher mit 1,25 Mol. Chloracetylchlorid + 1,5 Mol. n-Natronlauge unter starker Kühlung gekuppelt. Bei der Zugabe der berechneten Menge 5-fach normaler Salzsäure begann sofort die Ausscheidung des schön krystallisierenden Reaktionsproduktes, das nach 12-stündigem Stehen im Eisschrank abgesaugt wurde. F. = 132—133°. Nach einmaligem Auskrystallisieren aus Wasser wurde die optische Drehung bestimmt.

Optische Bestimmung.

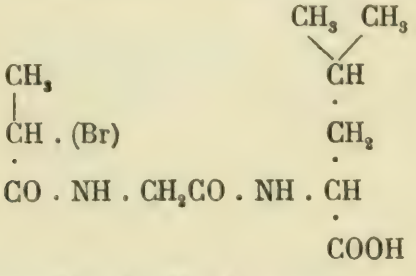
0,6228 g Substanz in absolutem Alkohol gelöst. Gesamtgewicht der Lösung 6,1036 g, d = 0,809, α im 5 cm-Rohr = — 0,57°; $[\alpha]_D^{20}$ = — 13,82°.

Die Amidierung erfolgte durch fünf Tage langes Stehenlassen bei Zimmertemperatur mit der 10fachen Menge 25%igen wässerigen Ammoniaks. Nach dem Eindampfen der ammoniakalischen Flüssigkeit im Vakuum zur Trockene wurde der Rückstand mit wenig heißem Wasser aufgenommen und die Lösung mit absolutem Alkohol bis zur bleibenden Trübung versetzt. Die bald entstandene, dicke weiße Krystallausscheidung wurde nach 12-stündigem Stehen in der Kälte abgesaugt.

Optische Untersuchung:

0,2281 g Substanz in Wasser gelöst; Gesamtgewicht der Lösung 7,3646 g; d = 1,008, α (im 5-cm-Rohr) = — 0,55°; $[\alpha]_D^{20}$ = — 35,23°.

d-α-Brompropionyl-glycyl-l-leucin:



6 g des Dipeptids (1 Mol.) wurden in 32 ccm (1 Mol.) n-Natronlauge gelöst und mit 6,5 g (1,2 Mol.) d-α-Brompropionylchlorid + 43 ccm (1,3 Mol.) n-Natronlauge gekuppelt. Beim vorsichtigen Übersäuren mit 5-fach n-Salzsäure gelangte ein farbloses harziges Produkt zur Ausscheidung, das behufs Be-

freiung von der eingeschlossenen Mutterlauge mit kaltem Wasser verrieben und damit gewaschen wurde. Das so behandelte Produkt erstarrte über Nacht im Exsikkator vollständig und wurde aus heißem Essigäther umkrystallisiert. Beim langsamen Verdunsten schieden sich schöne sternförmig angeordnete Nadeln ab, die sich sodann zu einem Brei vermehrten. Er wurde abgesaugt und von der Mutterlauge ausgewaschen. F. = 145° (unkorr.). Die Mutterlauge gab beim Versetzen mit Petroläther Krystalle von F. = 130°, die aus Essigäther nochmals umkrystallisiert, bei 145° schmolzen.

Die vom ursprünglichen Harz abdekantierte schwach salzsaure Lösung gab beim Kratzen der Gefäßwand in der Kälte eine weiße Krystallabscheidung, die sich über Nacht im Eisschrank stark vermehrte. Die abgesaugte Krystallmasse zeigte nach dem Trocknen F. = 147°.

Die Mutterlauge wurde unter vermindertem Druck bei 12 mm weiter eingedampft, wobei sich vom Kupplungsprodukt noch weitere Mengen (F. = 145—147°) abgeschieden haben. Gesamtausbeute: 7 g. Die nochmalige Umkrystallisation aus Essigester ergab makroskopische, konzentrisch gruppierte Nadeln oder Prismen von F. = 152° [konstant] (unkorr.). Unter dem Mikroskope zeigte ein aus absolutem Alkohol gewonnener Anteil schöne rosettenförmig angeordnete scharfkantige, dünne und lange Prismen.

Analysen:

0,1015 g Substanz: 0,1526 g CO_2, 0,0547 g H_2O.
0,1218 » » : 0,0691 » AgBr.
0,0982 » » : 6,30 ccm $^1/_{10}$-n-Schwefelsäure.

Berechnet für $C_{11}H_{19}O_4N_2Br$ (= 323,12):	Gefunden:
C 40,85%	41,00%
H 5,92%	6,03%
Br 24,74%	24,14%
N 8,67%	8,99%.

Löslichkeitsverhältnisse:

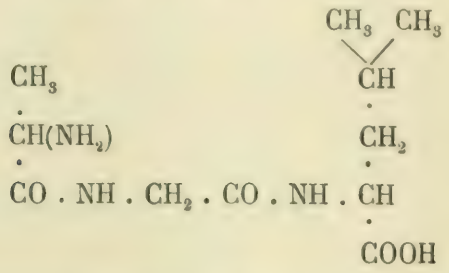
Essigäther : heiß leichtlöslich, kalt viel schwerer.
Petroläther: unlöslich.

Äther : kalt schwer, heiß etwas leichter.
Alkohol : sehr leicht löslich.
H_2O : schon in der Kälte ziemlich leicht löslich.

Optisches Verhalten:

0,4730 g Substanz in absolutem Alkohol gelöst: Gesamtgewicht der Lösung 6,1270 g, d = 0,810, α (im 5-cm-Rohr) = + 0,46°; $[\alpha]_D^{20}$ = + 14,71°.

d-Alanyl-glycyl-l-leucin.



5 g des Bromkörpers wurden unter genau den gleichen Bedingungen wie die vorher beschriebenen Produkte amidiert. Beim Versetzen der vom Ammoniak im Vakuum völlig befreiten konzentrierten Tripeptidlösung mit absolutem Alkohol entstand nach einiger Zeit im Eisschrank eine weiße krystallinische Abscheidung, die nach längerem Stehen abgesaugt und gewaschen wurde. Ausbeute 3 g. Geschmackloses Pulver. Es ist in kaltem Wasser sehr leicht löslich, unlöslich in absolutem Alkohol. Das Produkt gibt ausgesprochene Biuretreaktion.

Bei der Verdunstung einer wässerigen Lösung am Wasserbade krystallisiert das Tripeptid an der Oberfläche der Lösung in seidenglänzenden Nadeln aus, die abgesaugt und getrocknet F. = 243° unter Gasentwicklung und Zersetzung aufweisen (bei 235° leichte Bräunung, bei 238—240° Dunkelbraunfärbung).

Die Mutterlauge gibt beim vollständigen Verdunsten als Rückstand die gleich schmelzenden Krystalle.

Analysen:

0,1018 g Substanz: 0,1897 g CO_2, 0,0770 g H_2O.
0,0808 » » : 9,47 ccm $^1/_{10}$-n-H_2SO_4.

Berechnet für $C_{11}H_{21}O_4N_3$ (Mol.-Gew. = 259,19):	Gefunden:
C 50,93%	50,82%
H 8,16%	8,46%
N 16,21%	16,42%.

Optisches Verhalten:

0,2468 g Substanz in Wasser gelöst: Gesamtgewicht der Lösung 11,5777 g; d = 1,005; α (im 1-dm-Rohr) = — 0,24°; $[\alpha]_D^{20}$ = — 11,20°.

Kupferverbindung:

Sie wurde ebenso dargestellt, wie es bei den vorherigen Tripeptiden beschrieben wurde. Das Kupfersalz bildet eine blaue glasige Masse, die sich in Wasser mit ultramarinblauer Farbe auflöst. In absolutem Alkohol ist es schwer löslich, leichter in verdünntem.

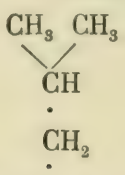
Analysen:

0,1157 g Substanz: 0,0204 g CuO = 14,09% Cu.

Vgl. den entsprechenden Wert der Kupferverbindung des Glycyl-d-alanyl-l-leucins.

d-Alanyl-l-leucyl-glycin.

l-Leucyl-glycin.[1])



(NH_2) . CH . CO . NH . CH_2 . COOH

8,1 g Glykokoll (1,2 Mol.) wurden in 106 ccm n-Natronlauge gelöst (1,2 Mol.) und mit 19 g d-α-Bromisocapronylchlorid (1 Mol.) und 115 ccm n-Natronlauge gekuppelt. Nach dem Übersäuren mit 5fach n-Salzsäure fiel ein hellgelbes zähes Öl aus, das nach dem Dekantieren der wässerigen Lösung mit Äther aufgenommen wurde. Die dekantierte Flüssigkeit wurde bei 12 mm eingedampft und der Rückstand ebenfalls mit Äther extrahiert. Der mit Magnesiumsulfat getrocknete ätherische Auszug wurde mit Petroläther versetzt, wobei wieder ein öliges Produkt zur Ausfällung gelangte. Nach Auflösen des Öls in

[1]) Emil Fischer, Synthese von Polypeptiden, XV., Ber. d. Deutsch. Chem. Gesellsch., Jg. 39, S. 2893, 1906.

heißem Chloroform schied sich beim Abkühlen ein farbloses krystallinisches Produkt vom F. = 117—118° ab (1,5 g). Die Krystalle erwiesen sich als zum größten Teile racemisiertes d-α-Bromisocapronyl-glycin, da sie $[\alpha]_D^{20} = +28{,}07°$ aufwiesen.

Die Chloroform-Mutterlauge wurde eingeengt und die sirupöse Lösung mit Petroläther gefällt. Anfangs fiel das Produkt ölig hinaus, wiederholte man jedoch diese Art der Umfällung, so erstarrte es schließlich zu einem krystallinischen Brei, der abgesaugt werden konnte. F. = 79—80° (12 g).

Die Reinigung erfolgte durch Auflösen in heißem Wasser und Verdunsten der von einem öligen Anteil abfiltrierten Lösung im Vakuum. Hierbei gelangten schöne weiße Krystalle zur Ausscheidung. F. = 82° [unkorr.] Optische Bestimmung 0,3558 g Substanz in absolutem Alkohol; Gesamtgewicht 12,2125 g; d = 0,801; α im 1-dm-Rohr = + 1,48°; $[\alpha]_D^{20} = +63{,}39°$.

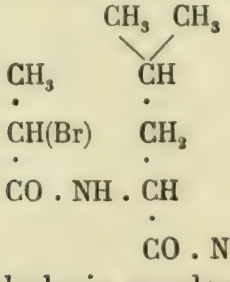
10 g des Bromkörpers wurden mit der 10-fachen Menge 25%igen Ammoniaks 5 Tage lang bei Zimmertemperatur amidiert. Die Isolierung des Dipeptids erfolgte nach der oben schon beschriebenen Silbersulfat-Methode. Das vom Baryumsulfatniederschlag abgetrennte Filtrat hinterließ beim Verdunsten im Vakuum einen Rückstand von 7 g, der in wenig Wasser gelöst wurde. Diese Lösung wurde am Wasserbade bis zur bleibenden Trübung mit Alkohol versetzt und der entstandene Krystallbrei abgesaugt (5 g).

Optische Untersuchung.

0,1356 g in Wasser gelöst; Gesamtgewicht der Lösung 6,7860 g. d = 1,005, α (im ½-dm-Rohr) = 0,85°;

$$[\alpha]_D^{20} = -84{,}56°.$$

α-d-Brompropionyl-l-leucyl-glycin:



5 g l-Leucyl-glycin wurden in 27 ccm (1 Mol.) n-Natronlauge gelöst und mit 5,7 g (1,3 Mol.) d-Brompropionylchlorid

und 40 ccm n-Natronlauge (1,5 Mol.) gekuppelt. Nach dem Übersäuren mit 5fach n-Salzsäure entstand nach einigem Stehen unter Eiskühlung ein farbloser krystallinischer Niederschlag, der nach 16-stündigem Stehen in der Kälte abgesaugt wurde. Ausbeute $5^1/_2$ g; F. = 153—154°. Die Reinigung wurde aus heißem Essigäther unternommen. Beim Verdunsten der heißen Lösung kommt der Körper in schneeweißen Krystallen heraus, die bei 154—155° konstant schmelzen (schmelzen zu einer klaren Flüssigkeit. Sintern 1° vorher). Aus heißem Alkohol auskrystallisiert, zeigt die Substanz unter dem Mikroskope schöne seeigelförmig zusammengewachsene feine Nadeln.

Durch Verdunstung des salzsauren Filtrats im Vakuum, Extraktion des Rückstandes mit siedendem Äther (zur Entfernung der Bromfettsäure) und mit siedendem Essigäther erhält man durch Eindunsten des Essigätherauszuges einen öligen Rückstand, der nicht zur Krystallisation gebracht werden konnte.

Löslichkeitsverhältnisse:

Äther : kalt und heiß schwer löslich.
Essigäther : kalt schwer, heiß leicht löslich.
Petroläther: unlöslich.
Alkohol : sehr leicht löslich.
H_2O : kalt schwer, heiß sehr leicht löslich.

Analysen:

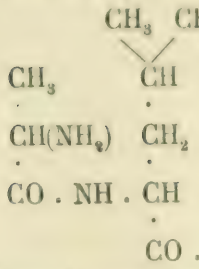
0,1438 g Substanz: 0,2157 g CO_2, 0,0774 g H_2O.
0,1309 » » : 0,0755 » AgBr.
0,0998 » » :6,05 ccm $^1/_{10}$-n-H_2SO_4.

Berechnet für $C_{11}H_{19}O_4N_2Br$ (= 323,12):		Gefunden:
C	40,85%	40,91%
H	5,92%	6,02%
Br	24,74%	24,54%
N	8,67%	8,49%.

Optisches Verhalten:

0,3102 g Substanz in absolutem Alkohol gelöst. Gesamtgewicht der Lösung: 6,0885 g, d = 0,806, α (im $^1/_2$-dm-Rohr) = — 0,51°; $[\alpha]_D^{20}$ = — 24,84°.

d-Alanyl-l-leucyl-glycin:



4,2 g des Bromkörpers wurden in gewohnter Weise amidiert. Die mit überschüssigem Alkohol versetzte konzentrierte wässerige Tripeptidlösung begann nach einiger Zeit bei Eiskälte die weiße krystallinische Masse des Tripeptids auszuscheiden. Nach 24 Stunden wurde abgesaugt und gewaschen. Ausbeute: 2,7 g schneeweißes, geschmackloses Pulver. Das Tripeptid ist in kaltem Wasser sehr leicht löslich, unlöslich in absolutem Alkohol. Beim Eindunsten der wässerigen Lösung erhält man wunderschöne krystallinische Nadeln, die so gruppiert sind, wie bei den früher beschriebenen Tripeptiden angegeben wurde. Diese Krystalle haben F. = 246—47° und schmelzen unter Zersetzung und Dunkelbraunfärbung (bei 235° fängt die Braunfärbung an). Die eingedampfte Mutterlauge hinterläßt Krystalle vom genau gleichen Schmelzpunkt.

Das Tripeptid gibt sehr starke Biuretreaktion.

Analysen:

0,1073 g Substanz: 0,1990 g CO_2, 0,0785 g H_2O.
0,0834 » » : 9,60 ccm $^1/_{10}$-n-H_2SO_4.

Berechnet für $C_{11}H_{21}O_4N_3$ (= 259,19):		Gefunden:
C	50,93%	50,58%
H	8,16%	8,18%
N	16,21%	16,13%

Optisches Verhalten.

0,2269 g Substanz in Wasser gelöst. Gesamtgewicht der Lösung: 10,8426 g, d = 1,005, α (im 1-dm-Rohr) = — 0,64°; $[\alpha]_D^{20}$ = — 30,43.

Kupferverbindung.

Die Darstellung erfolgte in gewohnter Weise. Die wässerige Lösung der graublauen glasigen Masse ist violettrot gefärbt.

In absolutem Alkohol schwer löslich, in verdünntem Alkohol leichter.

Analyse:

0,1040 g Substanz: 0,0184 g CuO = 14,13% Cu.

Vgl. Kupferverbindung von Glycyl-d-alanyl-l-leucin.

l-Leucyl-d-alanyl-glycin.

d-Alanyl-glycin:[1])

CH_3

$CH . NH_2$

$CO . NH . CH_2 . COOH$

10,4 g Glykokoll (1,3 Mol.) wurden in 138 ccm n-Natronlauge (1,3 Mol.) gelöst und mit 18 g d-Brompropionylchlorid (1 Mol.) und 138 ccm n-Natronlauge in der gewöhnlichen Weise gekuppelt. Nach Eindampfen der übersäuerten Lösung bei 12 mm Druck wurde der Rückstand mit siedendem Äther erschöpfend ausgekocht und der getrocknete Auszug des letzteren eingedunstet. Die konzentrierte ätherische Lösung gab nach dem Versetzen mit Petroläther eine ölige Fällung, die im Eisschrank nach wenigen Minuten krystallinisch zu erstarren begann. Nach 12 Stunden erstarrte das Öl vollständig und wurde mit Petroläther gewaschen und abgesaugt (15 g).

Optische Bestimmung:

0,2079 g Substanz in Wasser gelöst. Gesamtgewicht der Lösung: 6,0022 g, d = 1,005, α (im 2-cm-Rohr) = + 0,26°, $[\alpha]_D^{20}$ = + 37,33°.

Die Amidierung erfolgte mit der 10fachen Menge 25%igen wässerigen Ammoniaks (5 Tage, Zimmertemperatur).

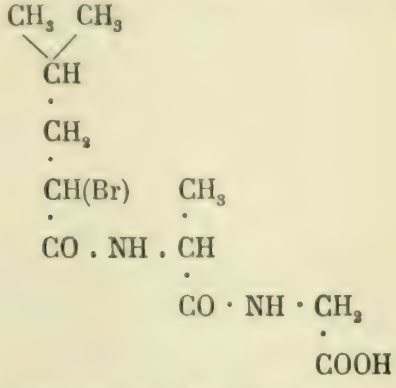
Das Dipeptid wurde nach der Silbersulfatmethode isoliert. Das sich hierbei ergebende Filtrat vom Baryumsulfatniederschlag wurde nach dem Verdunsten bei 12 mm mit wenig Wasser aufgenommen und die Lösung mit Alkohol versetzt. Bald begann die Krystallisation des Dipeptids (8,5 g).

[1]) E. Fischer, Ber. d. Deutsch. Chem. Gesellsch., Jg. 41, S. 850 (1908).

Optische Bestimmung.

0,1930 g Substanz in Wasser gelöst. Gesamtgewicht der Lösung: 6,2240 g, d = 1,004, α (im ½-dm-Rohr) = + 0,75°; $[\alpha]_D^{20}$ = + 48,33°.

α-d-Bromisocapronyl-d-alanyl-glycin:



5 g des Dipeptids (1 Mol.) wurden in 34,3 ccm (1 Mol.) n-Natronlauge gelöst und mit 9,2 g (1,25 Mol.) d-α-Bromisocapronylchlorid und 51 ccm (1,5 Mol.) n-Natronlauge gekuppelt. Am Schluß wurde von wenig öliger Masse abfiltriert und mit 5-fach normaler Salzsäure vorsichtig übersäuert. Die sofort entstandene dicke weiße krystallinische Ausscheidung wurde nach 12-stündigem Stehen im Eisschrank abgesaugt und ausgewaschen. Zur Reinigung wurde der Körper aus siedendem Essigäther umkrystallisiert. Beim langsamen Verdunsten der Lösung scheidet sich das Produkt in schönen Krystallen aus, die abgesaugt und mit Petroläther gewaschen wurden. Bei 129° schmilzt der Körper zu einer klaren Flüssigkeit (aus Essigäther konst.). In heißem Wasser gelöst, scheidet er sich beim Erkalten in hübschen sternförmig gruppierten Nädelchen aus, die ebenfalls bei 129° (unkorr.) schmelzen (Ausbeute 7 g).

Nach vollständigem Verdunsten des salzsauren Filtrats im Vakuum und Ausziehen des Rückstandes mit siedendem Essigäther und Fällen des getrockneten und eingeengten Auszuges mit Petroläther erhält man eine krystallinische Ausfällung. Da dieselbe trotz längeren Waschens mit Petroläther klebrig blieb, so wurde sie aus heißem Wasser umkrystallisiert. Beim Verdunsten der wässerigen Lösung entstanden wenige

Zentigramm Krystalle, die abgesaugt, gewaschen und auf Ton gepreßt F. = 197° besaßen und 25° höher unter Braunfärbung zersetzt wurden.

Dieses Produkt wurde nicht weiter untersucht.

Löslichkeitsverhältnisse:

Äther : kalt schwer, heiß etwas leichter löslich.
Essigäther : kalt ziemlich schwer, heiß leicht löslich.
Petroläther: unlöslich.
Alkohol : leicht löslich.
H_2O : kalt recht schwer, heiß leicht löslich.

Analysen:

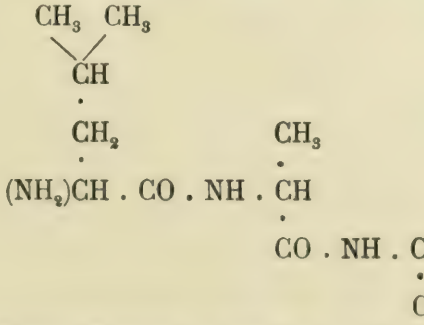
0,1017 g Substanz: 0,0604 g AgBr.
0,1373 » » : 0,2050 g CO_2, 0,0735 g H_2O.
0,1124 » » : 6,99 ccm $^1/_{10}$-n-H_2SO_4.

Berechnet für $C_{11}H_{19}O_4N_2Br$ (= 323,12):		Gefunden:
C	40,85%	40,72%
H	5,92%	5,99%
Br	24,74%	25,26%
N	8,67%	8,71%

Optisches Verhalten:

0,3143 g Substanz in absolutem Alkohol gelöst. Gesamtgewicht der Lösung: 6,3890 g, d = 0,806; α (in $^1/_2$-dm-Rohr) = − 0,05°; $[\alpha]_D^{20}$ = − 2,52°.

l-Leucyl-d-alanyl-glycin:



4 g des Bromkörpers wurden mit der 5-fachen Menge 25%igen Ammoniaks 5 Tage lang bei Zimmertemperatur amidiert. Beim Verdunsten der Lösung im Vakuum begann sehr

bald die weiße krystallinische Abscheidung des Tripeptids, die sich beim weiteren Eindampfen stark vermehrte. Der Rückstand wurde hierauf in heißem Wasser gelöst und die Lösung mit überschüssigem Alkohol versetzt, worauf sogleich eine voluminöse Ausscheidung begann. Dieselbe wurde nach 12-stündigem Stehen bei Eiskälte abgesaugt.

Das Tripeptid ist in kaltem Wasser schwer löslich, leichter in der Hitze. In absolutem Alkohol löst es sich nicht. Beim Eindunsten der wässerigen Lösung erhält man sehr schöne, makroskopisch sichtbare feine Nadeln, die sich an der Oberfläche ausscheiden. Dieselben färben sich bei 244° bräunlich und schmelzen unter starker Braunfärbung und Zersetzung bei 252—53°. Die Mutterlauge gibt beim vollständigen Eindunsten einen Rückstand mit dem identischen Schmelz- resp. Zersetzungspunkt.

Das Tripeptid gibt im Gegensatz zu den vorigen 4 Isomeren eine sehr schwache (gleiche Bedingungen!) Biuretprobe.

Analysen:

0,0748 g Substanz: 0,1379 g CO_2, 0,0552 g H_2O.
0,1160 » » : 13,53 ccm n/10-H_2SO_4.
0,0906 » » : 8,90 » N nach Van Slyke (735 mm, 24°).

Berechnet für $C_{11}H_{21}O_4N_3$ (= 259,19);		Gefunden:
C	50,93%	50,28%
H	8,16%	8,25%
N	16,21%	16,34%
Amino-N:	5,40%	5,45%

Optisches Verhalten:

0,2389 g Substanz in Wasser gelöst. Gesamtgewicht der Lösung = 20,7410 g, d = 1,003; α (im 1-dm-Rohr) = — 0,20°; $[\alpha]_D^{20}$ = — 17,31°.

Kupferverbindung.

Dieselbe ist eine blaugefärbte amorphe, glasige Masse, die aber im Gegensatz zu den 4 anderen Isomeren in absolutem Alkohol sehr leicht löslich ist. Diese Lösung ist, wie

auch die wässerige, tiefblau gefärbt. Beim Versetzen mit Äther gibt die alkoholische Lösung eine flockige Ausscheidung, die besonders analysiert wurde.

Analysen: Gefunden:

0,1137 g Subst.: 0,0242 g CuO = 17,01 % Cu.

0,1712 » » : 13,29 ccm $^{n}/_{10}$-H_2SO_4 (Kjeldahl) = 10,87 % N.

Das Atomverhältnis stellt sich somit als 3 N : 1 Cu heraus.

Für $C_{11}H_{21}O_4N_3 \cdot CuO \cdot 2\,H_2O$ (= 374,83 g). Ber.: 16,98 % Cu.
11,21 % N.

Das aus der absoluten alkoholischen Lösung mit Äther ausgefällte Produkt gab folgendes Resultat:

0,1404 g Substanz: 0,0324 g CuO = 18,44 % Cu.

Für $C_{11}H_{21}O_4N_3 \cdot CuO$ (= 338,79). Berechnet: 18,77 % Cu.

Darstellung der zu den biologischen Versuchen verwendeten Fermentlösungen.

I. Hefe — Mazerationssaft.[1])

50 g Trockenhefe wurden mit der dreifachen Wassermenge verrührt und das Gemenge 2—3 Stunden hindurch im Brutschrank bei 37° aufbewahrt. Durch Filtration (Faltenfilter) wurde ein klarer Saft gewonnen, der mit dem gleichen Volumen physiologischer Kochsalzlösung verdünnt wurde (Saft A). Da dieser Saft noch ziemlich stark rotbraun gefärbt war und bei den optischen Versuchen im Brutschrank sehr bald ganz undurchsichtig wurde, so erwies es sich vorteilhafter, das ursprüngliche Filtrat mit der 2,5fachen Wassermenge zu verdünnen (Saft B). Der so gewonnene Saft ist außerordentlich aktiv. Zu manchen Zwecken wurde Saft B mit noch 1 Volumen physiologischer Kochsalzlösung (7 ‰) verdünnt: Saft B'.

II. Darstellung von Leberpreßsaft.

Eine ganz frische Schweineleber wurde, wie üblich, mit Sand zermahlen, mit Kieselgur vermengt und bei 250 Atmosphären ausgepreßt. Der durch ein braunes Gerinnsel und Fett-

[1]) Vgl. hierzu: A. v. Lebedew, Darstellung des aktiven Hefenpreßsaftes durch Maceration. Diese Zeitschrift, Bd. 73, S. 447 (1911).

tröpfchen getrübte Preßsaft wurde mit dem 3-fachen Volumen physiologischer Kochsalzlösung verdünnt und gleichzeitig durch eine Anzahl von Filtern filtriert. Auf diese Weise kommt man schneller zum Ziel als durch Zentrifugieren des Saftes. Das braunrot gefärbte Filtrat wurde nach 48-stündigem Verweilen im Brutschrank von koagulierten Eiweißkörpern durch eine Tonkerze abgetrennt. Der so gewonnene Saft (Saft L) war vollkommen klar und beim Aufbewahren im Eisschrank ziemlich haltbar.

III. Darstellung von aktiviertem Pankreassaft.

Es wurde eine 5%ige wässerige Lösung eines angeblich zymogenen,[1]) unter stark vermindertem Druck bei niederer Temperatur eingedampften Pankreassaftes, den wir der Güte des H. Prof. Dr. E. S. London, St. Petersburg, verdanken, bereitet (Saft P) und hierzu 1/2% Darmsaftfermentpräparat gefügt. Die filtrierte Lösung stellt Saft D vor.

Bei Spaltungsversuchen mit Darmsaft für sich gelangte eine 1/2%ige wässerige Lösung des Darmsaftpräparates zur Anwendung (Saft D').

IV. Gewinnung des Pankreaspreßsaftes aus der Pankreasdrüse eines Diabetikers.

Aus der stark bronzebraun gefärbten Drüse wurde bei 300 Atmosphären ein Preßsaft bereitet. Der 25 ccm betragende Saft war selbst nach dem Verdünnen mit dem gleichen Volumen physiologischer Kochsalzlösung tief fuchsinrot gefärbt. Die so verdünnte Lösung schied beim Stehen über die Nacht im Eisschrank ein rotbraunes Gerinnsel aus (offenbar das Pigment der pathologisch veränderten Drüse). Nach dem Filtrieren durch eine Hartfilterbatterie wurde ein klarer Saft erhalten, der aber noch so tief gefärbt war, daß er mit dem gleichen Volumen Kochsalzlösung verdünnt werden mußte (Saft K).

V. Gewinnung des Preßsaftes aus einer normalen Pankreasdrüse.

Die 78 g schwere Drüse gab beim Pressen bis 250 Atmosphären 11 ccm Saft ab und zwischen 250 und 300 Atmosphären 20 ccm.

[1]) Die Lösung erwies sich als aktiv.

Der Saft wurde mit dem gleichen Volumen einer physiologischen Kochsalzlösung verdünnt und durch gehärtete Filter filtriert. Tonkerze war nicht anwendbar, weil beim Filtrieren durch diese der Saft seiner Aktivität beraubt wurde.

Zur Verwendung kam der bei höherem Drucke ausgepreßte Saft (K').

VI. Gewinnung des Preßsaftes aus der Pankreasdrüse eines an Morbus Basedowii gestorbenen Patienten.

Die Drüse wies eine solche Saftarmut auf, daß ein Verreiben mit 50 ccm physiologischer Kochsalzlösung erforderlich war. Die weitere Behandlung des Saftes geschah wie sub V und VI (Saft K'').

$$[\alpha]_D^{20} = \underbrace{\underbrace{-35{,}09^\circ}_{} \underbrace{+10^\circ}_{}}_{[\alpha]_D^{20} = -59{,}04^\circ}$$

Spaltung von Glycyl-l-leucyl-d-alanin.

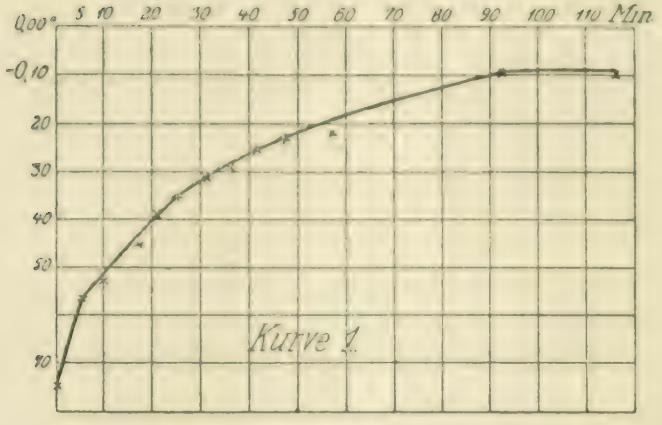
Stammlösung: 0,2120 g in 10 ccm physiologischer Kochsalzlösung (7‰). (0,0817 Mol. im Liter.)

Mit Hefesaft B.

a) 5 ccm Stammlösung (= 0,1060 g) + 2,5 ccm Saft B + 0,5 ccm physiologische Kochsalzlösung.

b) 2,5 ccm Saft B + 5,5 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr.	b) α = konstant — 0,02°.
0	— 0,75°	— 0,73°	
5	— 0,56°	— 0,54°	
10	— 0,53°	— 0,51°	
17	— 0,45°	— 0,43°	
21	— 0,39°	— 0,37°	
25	— 0,35°	— 0,33°	
31	— 0,31°	— 0,29°	(Hierzu Kurve 1.)
36	— 0,29°	— 0,27°	
41	— 0,25°	— 0,23°	
47	— 0,23°	— 0,21°	
57	— 0,22°	— 0,20°	
93	— 0,09°	— 0,07°	
116	— 0,10°	— 0,08° (konst.)	

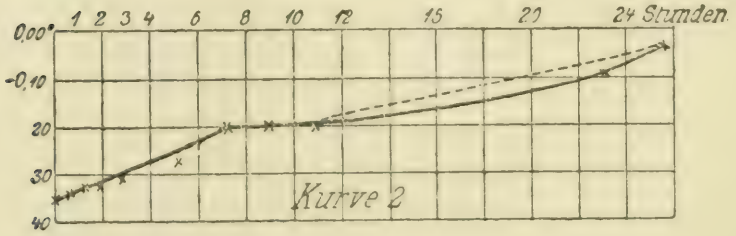


Mit Leberpreßsaft L.

a) 2 ccm Stammlösung (= 0,0424 g) + 1 ccm Saft L + 5 ccm physiologische Kochsalzlösung.

b) 1 ccm Saft L + 7 ccm physiologische Kochsalzlösung.

a) Stunden		b) α = konstant 0,00°.
0	— 0,35°	
0,50	— 0,34°	
1,25	— 0,32°	
1,80	— 0,32°	
2,75	— 0,31°	
5,25	— 0,27°	(Hierzu Kurve 2.)
7,25	— 0,19°	
8,75	— 0,21°	
10,75	— 0,20°	
23,00	— 0,09°	
25,50	— 0,03° (konst.)	

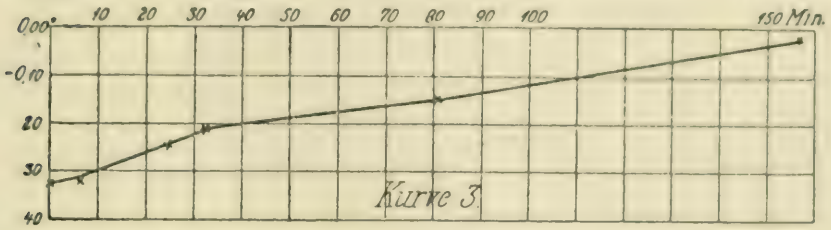


Mit aktiviertem Pankreassaft D.

a) 2 ccm Stammlösung (0,0424 g) + 2 ccm Saft D + 4 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft D + 6 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr.	b)
0	— 0,72°	— 0,32°	
6	— 0,72°	— 0,32°	
25	— 0,65°	— 0,25°	
32	— 0,61°	— 0,21°	— 0,40°
60	— 0,59°	— 0,19°	
81	— 0,55°	— 0,15°	
157	— 0,42°	— 0,02°	
228	— 0,38°	— 0,02° (konst.)	— 0,36°



Mit nicht aktiviertem Pankreassaft P.

a) 2 ccm Stammlösung (= 0,0424 g) + 2 ccm Saft P + 4 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft P + 6 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr.	b)
0	— 0,61°	— 0,28°	— 0,33°
7	— 0,61°	— 0,28°	— 0,33°
87	— 0,59°	— 0,27°	— 0,32°
350	— 0,48°	— 0,13°	— 0,35°
480	— 0,36°	— 0,03°	— 0,33°

Mit Darmsaft D′.

a) 2 ccm Stammlösung (0,0424 g) + 2 ccm Saft D′ + 4 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft D′ + 6 ccm physiologische Kochsalzlösung (optisch inaktiv).

a) Minuten	
0	— 0,30°
13	
168	

Lösung a) wurde hierauf aufgekocht und vom ausgefallenen Eiweiß abfiltriert.

c) 5 ccm des Filtrates + 2 ccm Saft P + 1 ccm physiologische Kochsalzlösung. d) 2 ccm Saft P + 6 ccm physiologische Kochsalzlösung.

c) Stunden	unkorr.	korr.	d)
0	— 0,54°	— 0,22°	— 0,32°
0,37	— 0,58°	— 0,24°	— 0,34°
2,00	— 0,47°	— 0,16°	— 0,31°
4,00	— 0,44°	— 0,12°	— 0,32°
17,00	— 0,36°	— 0,05°	— 0,31°

Diese letzteren Versuche zeigen, daß die Wirkung des im Darmsaft enthaltenen Aktivators nicht auf einer Veränderung des Substrates durch den

letzteren beruht, da sonst die Spaltungsgeschwindigkeit des mit dem Aktivator längere Zeit in Berührung gewesenen Tripeptides erhöht sein müßte. (Vgl. Kurve 3.)

$$[\alpha]_D^{20} = \frac{-50^\circ \quad -17{,}21^\circ}{[\alpha]_D^{20} = -89{,}86^\circ}$$

Spaltung von Glycyl-d-alanyl-l-leucin.

Stammlösung: 0,2276 g in 10 ccm physiologischer Kochsalzlösung. (0,0878 Mol. im Liter.)

Mit Hefesaft B.

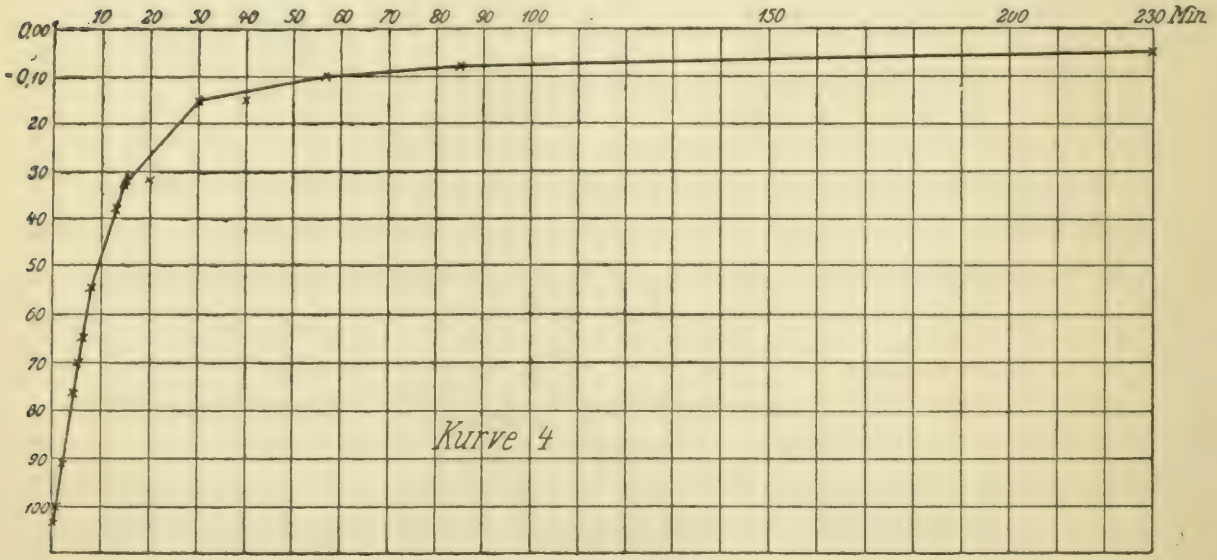
a) 5 ccm Stammlösung (= 0,1138 g) + 2,5 ccm Saft B + 0,5 ccm physiologische Kochsalzlösung.

b) 2,5 ccm Saft B + 5,5 ccm physiologische Kochsalzlösung.

b) α = konstant $-0{,}02^\circ$.

a) Minuten	unkorr.	korr.
0	$-1{,}05^\circ$	$-1{,}03^\circ$
1	$-1{,}00^\circ$	$-0{,}98^\circ$
2	$-0{,}93^\circ$	$-0{,}91^\circ$
4	$-0{,}78^\circ$	$-0{,}76^\circ$
5	$-0{,}72^\circ$	$-0{,}70^\circ$
6	$-0{,}67^\circ$	$-0{,}65^\circ$
8	$-0{,}57^\circ$	$-0{,}55^\circ$
13	$-0{,}40^\circ$	$-0{,}38^\circ$
15	$-0{,}35^\circ$	$-0{,}33^\circ$
16	$-0{,}34^\circ$	$-0{,}32^\circ$
20	$-0{,}34^\circ$	$-0{,}32^\circ$
30	$-0{,}17^\circ$	$-0{,}15^\circ$
40	$-0{,}17^\circ$	$-0{,}15^\circ$
57	$-0{,}12^\circ$	$-0{,}10^\circ$
85	$-0{,}10^\circ$	$-0{,}08^\circ$
230	$-0{,}07^\circ$	$-0{,}05^\circ$ (konst.)

(Hierzu Kurve 4.)



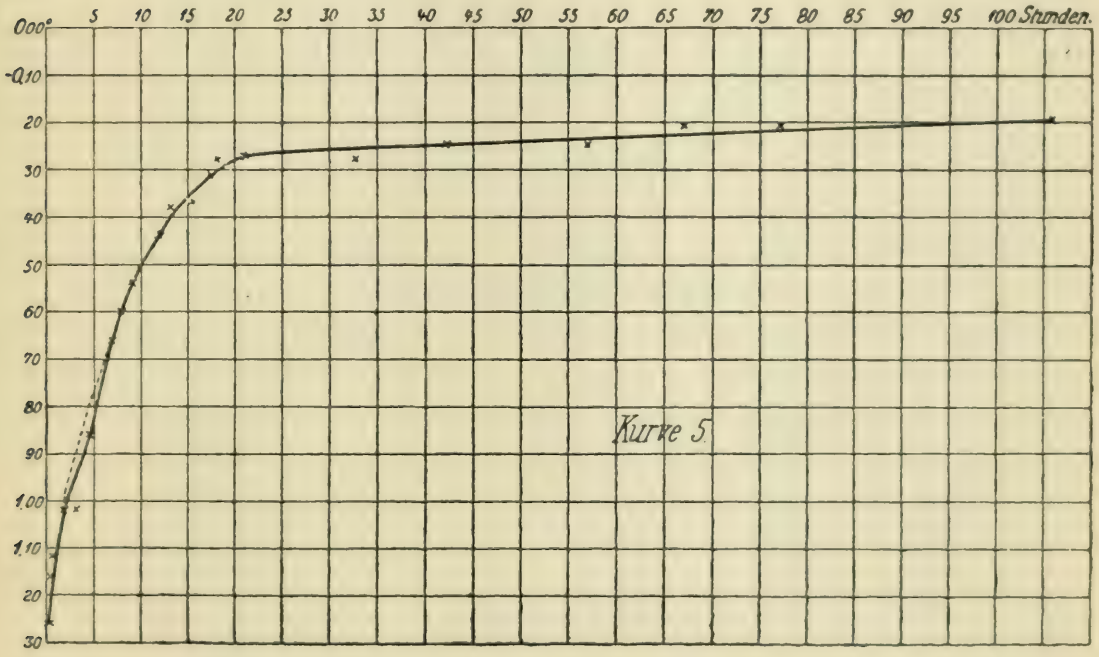
Mit Leberpreßsaft L.

a) 5 ccm Stammlösung (= 0,1138 g) + 1 ccm Saft L + 2 ccm physiologische Kochsalzlösung.

b) 1 ccm Saft L + 7 ccm physiologische Kochsalzlösung (konstant optisch inaktiv).

a) Stunden		Stunden	
0	— 1,26°	12,10	— 0,44°
0,33	— 1,16°	13,10	— 0,38°
0,75	— 1,12°	15,50	— 0,37°
1,60	— 1,02°	17,50	— 0,31°
2,30	— 1,02°	18,80	— 0,28°
2,90	— 1,02°	20,90	— 0,27°
4,50	— 0,86°	32,65	— 0,28°
6,90	— 0,66°	42,15	— 0,25°
8,00	— 0,60°	57,15	— 0,25°
Über die Nacht im Eis-		66,75	— 0,21°
schrank aufbewahrt:		82,40	— 0,21°
8,00	— 0,60°	105,65	— 0,19°
9,20	— 0,54°	154,15	— 0,08°

(Hierzu Kurve 5.)

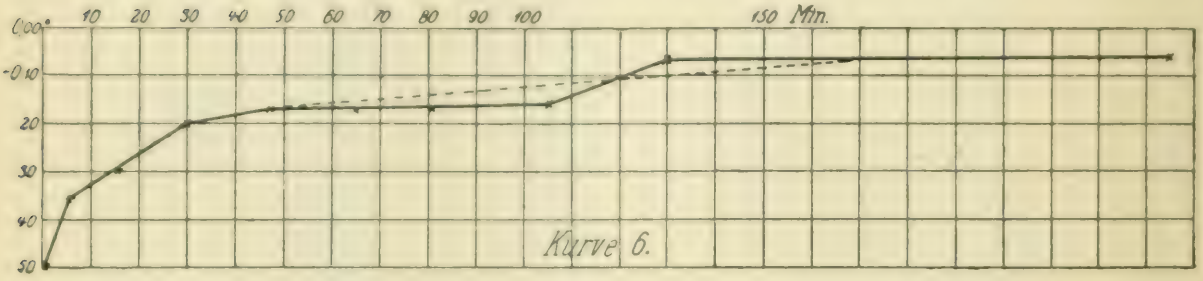


Mit aktiviertem Pankreassaft D.

a) 2,5 ccm der Stammlösung (= 0,0569 g) + 2 ccm Saft D + 3,5 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft D + 6 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr.	b)
0	— 0,84°	— 0,49°	— 0,35°
5	— 0,70°	— 0,35°	— 0,35°
15	— 0,63°	— 0,30°	— 0,33°
(Hierzu 30	— 0,54°	— 0,20°	— 0,34°
Kurve 6.) 47	— 0,47°	— 0,17°	— 0,30°
65	— 0,49°	— 0,18°	— 0,31°
81	— 0,48°	— 0,17°	— 0,31°
105	— 0,46°	— 0,16°	— 0,30°



$$[\alpha]_D^{20} = +50{,}2° - 35{,}09°$$

$$[\alpha]_D^{20} = -11{,}20°$$

Spaltung von d-Alanyl-glycyl-l-leucin.

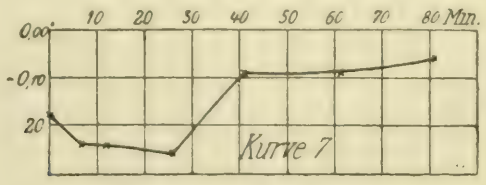
Stammlösung: 0,1991 g in 10 ccm physiologischer Kochsalzlösung (0,0768 Mol. im Liter.)

Mit Hefesaft B.

a) 5 ccm Stammlösung (= 0,0995 g) + 2,5 ccm Saft B + 0,5 ccm physiologische Kochsalzlösung.

b) 2,5 ccm Saft B + 5,5 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr.	b) α = konstant — 0,02°.
0	— 0,20°	— 0,18°	
7	— 0,26°	— 0,24°	
12	— 0,26°	— 0,24°	
26	— 0,28°	— 0,26°	(Hierzu Kurve 7.)
41	— 0,11°	— 0,09°	
61	— 0,11°	— 0,09°	
81	— 0,08°	— 0,06°	konstant.



Mit Leberpreßsaft L.

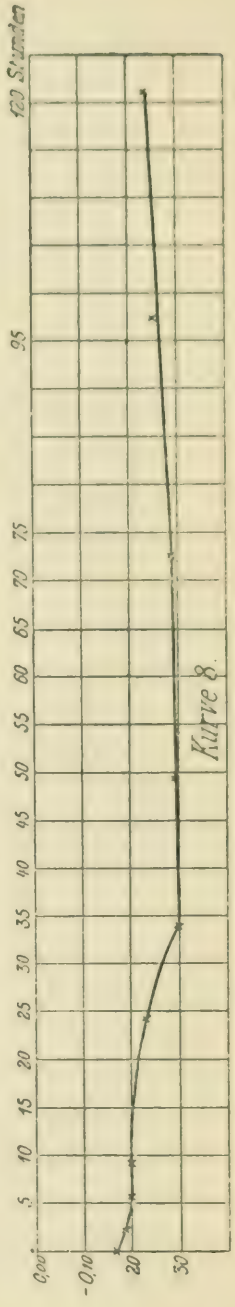
a) 5 ccm Stammlösung (= 0,0995 g) + 1 ccm Saft L + 2 ccm physiologische Kochsalzlösung.

b) 1 ccm Saft L + 7 ccm physiologische Kochsalzlösung.

(Konstant optisch inaktiv.)

a) Stunden		Stunden	
2,00	— 0,19°	49,33	— 0,29°
5,50	— 0,20°	72,50	— 0,29°
9,00	— 0,20°	97,33	— 0,25°
24,20	— 0,23°	121,00	— 0,23°
33,50	— 0,30°	147,33	— 0,19°

Kurve 8.



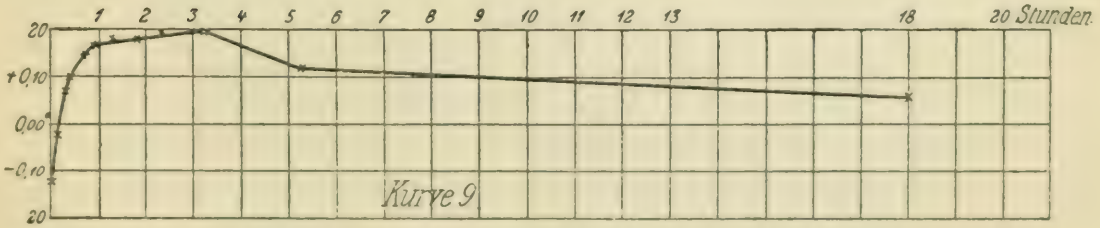
Mit aktiviertem Pankreassaft D.

a) 5 ccm Stammlösung (= 0,0995 g) + 2 ccm Saft D + 1 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft D + 6 ccm physiologische Kochsalzlösung.

a) Stunden	unkorr.	korr.	b)
0	— 0,41°	— 0,12°	
0,15	— 0,31°	— 0,02°	
0,30	— 0,22°	+ 0,07°	
0,43	— 0,19°	+ 0,10°	konstant
0,70	— 0,14°	+ 0,15°	= — 0,29°
0,93	— 0,12°	+ 0,17°	
1,35	— 0,11°	+ 0,18°	
1,85	— 0,12°	+ 0,17°	
2,35	— 0,08°	+ 0,19°	— 0,27°
3,25	— 0,09°	+ 0,20°	— 0,29°
5,25	— 0,09°	+ 0,12°	— 0,21°
18,05	— 0,16°	+ 0,06°	— 0,22°
21,15	— 0,18°	+ 0,03°	— 0,21°
25,25	— 0,18°	+ 0,03°	— 0,21°
27,75	— 0,18°	+ 0,03°	— 0,21°
42,25	— 0,17°	+ 0,03°	— 0,20°
51,45	— 0,17°	+ 0,01°	— 0,18°
66,25	— 0,23°	— 0,03°	— 0,20°

(Hierzu Kurve 9.)



$$[\alpha]_D^{20} = \underbrace{\underbrace{-17{,}21}_{} \quad \underbrace{+85{,}99}_{}}_{[\alpha]_D^{20} -30{,}41}$$

Spaltung von d-Alanyl-l-leucyl-glycin.

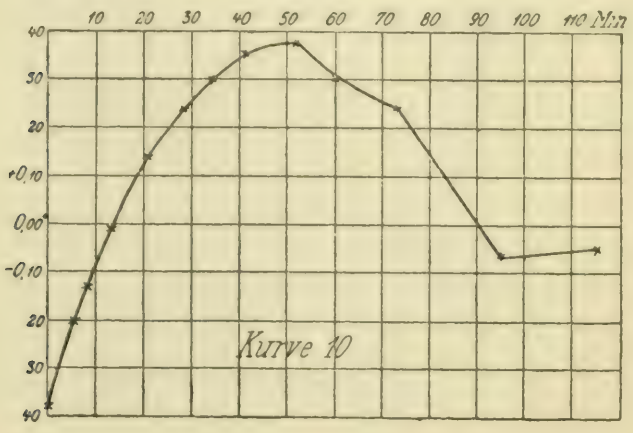
Stammlösung: 0,2040 g in 10 ccm physiologischer Kochsalzlösung (= 0,0787 Mol. im Liter).

Mit Hefesaft B.

a) 5 ccm Stammlösung (= 0,1020 g) + 2,5 ccm Saft B + 0,5 ccm physiologische Kochsalzlösung.

b) 2,5 ccm Saft B + 5,5 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr. — 0,38° (ber.)	b) α = konstant — 0,02°
0			
5	— 0,22°	— 0,20°	
8	— 0,15°	— 0,13°	
13	— 0,03°	— 0,01°	
20	+ 0,12°	+ 0,14°	
28	+ 0,22°	+ 0,24°	
33	+ 0,28°	+ 0,30°	
41	+ 0,34°	+ 0,36°	(Hierzu Kurve 10.)
51	+ 0,36°	+ 0,38°	
60	+ 0,28°	+ 0,30°	
73	+ 0,22°	+ 0,24°	
95	— 0,09°	— 0,07°	
115	— 0,07°	— 0,05°	

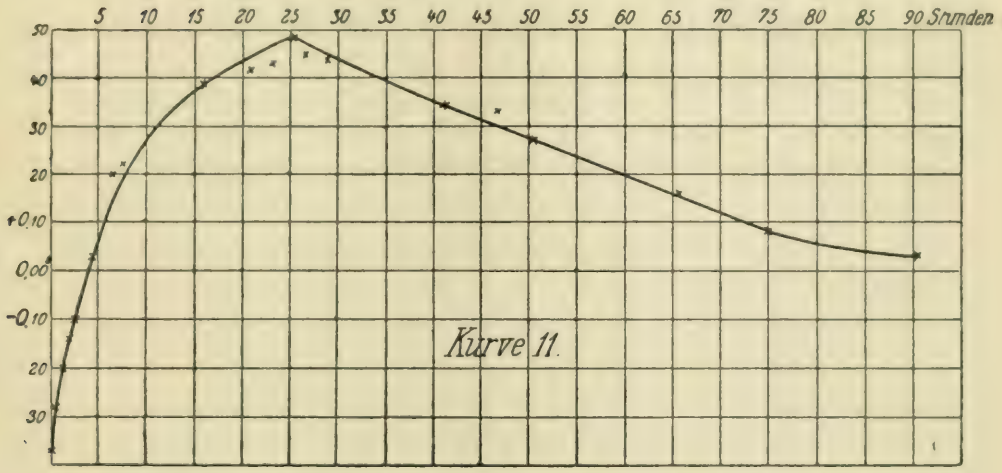


Mit Leberpreßsaft L.

a) 5 ccm Stammlösung (= 0,1020 g) + 1 ccm Saft L + 2 ccm physiologische Kochsalzlösung.

b) Lebersaft L ist konstant optisch inaktiv.

a) Stunden		Stunden	
0	− 0,37°	20,80	+ 0,42°
0,50	− 0,28°	23,25	+ 0,43°
1,40	− 0,20°	25,33	+ 0,49°
2,00	− 0,14°	26,75	+ 0,45°
2,60	− 0,10°	28,90	+ 0,44°
(Hierzu 4,40	+ 0,03°	41,23	+ 0,34°
Kurve 11.) 6,66	+ 0,20°	46,70	+ 0,33°
7,66	+ 0,22°	50,50	+ 0,27°
15,80	+ 0,39°	65,70	+ 0,16°
16,70	+ 0,36°	75,00	+ 0,08°
18,30	+ 0,36°	90,75	+ 0,03°
19,60	+ 0,39°		

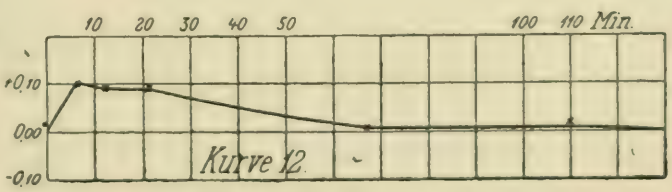


Die folgenden 2 Versuche wurden mit Hefesaft B bei größeren Verdünnungen ausgeführt. Sie ergeben die Unabhängigkeit des Ortes der Spaltung von der Konzentration.

a) 2,5 ccm Stammlösung (= 0,0510 g) + 2,5 ccm Saft B + 3 ccm physiologische Kochsalzlösung. (0,0197 Mol. im L.)

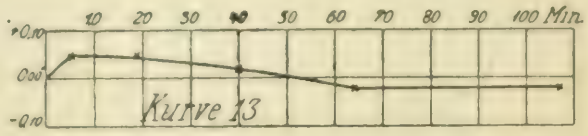
b) wie oben = konst. − 0,02°.

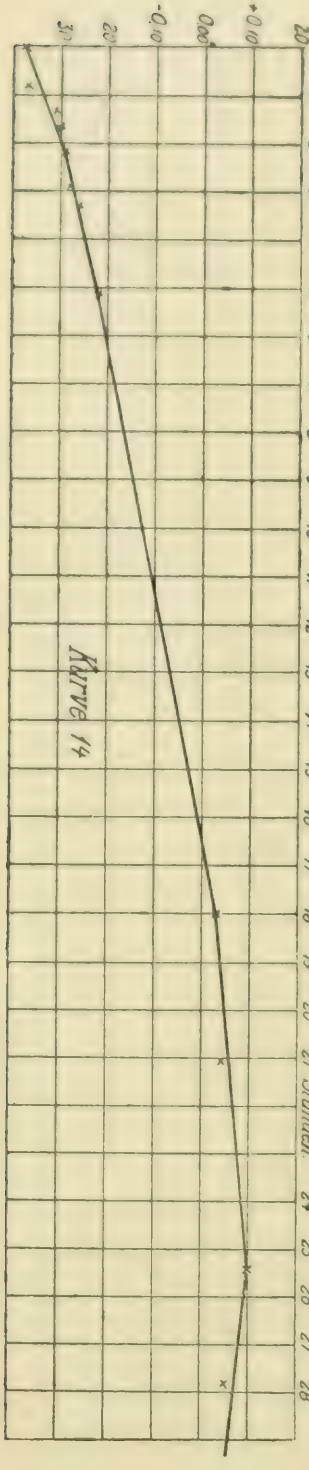
a) Minuten	korr.
0	0,00°
6	+ 0,12°
12	+ 0,11°
21	+ 0,11°
42	+ 0,02°
66	+ 0,01°
110	+ 0,02°
180	− 0,01°



a) 1,25 ccm Stammlösung (= 0,0255 g) + 2,5 ccm Saft B + 4,25 ccm physiologische Kochsalzlösung. 0,0098 Mol. im L.)
b) wie oben.

a) Minuten	korr.	
0	—	
5	+ 0,05°	(Hierzu Kurve 13.)
18	+ 0,05°	
40	+ 0,02°	
64	— 0,02°	
107	— 0,02°	





Mit aktiviertem Pankreassaft D.

a) 5 ccm Stammlösung + 2 ccm Saft D + 1 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft D + 6 ccm physiologische Kochsalzlösung.

a) Stund.	unkorr.	korr.	b)	
0	— 0,66°	— 0,37°	konstant = — 0,29°	
0,09	— 0,68°	— 0,39°		
0,20	— 0,68°	— 0,39°		
0,40	— 0,67°	— 0,38°		
0,80	— 0,67°	— 0,38°		
1,30	— 0,60°	— 0,31°		
1,72	— 0,59°	— 0,30°		
2,20	— 0,56°	— 0,29°	— 0,27°	
2,88	— 0,57°	— 0,28°	— 0,29°	(Hierzu
3,30	— 0,55°	— 0,26°	— 0,29°	Kurve 14.)
5,13	— 0,44°	— 0,23°	— 0,21°	
18,00	— 0,18°	+ 0,04°	— 0,22°	
21,10	— 0,17°	+ 0,04°	— 0,21°	
25,50	— 0,11°	+ 0,10°	— 0,21°	
27,80	— 0,16°	+ 0,05°	— 0,21°	
42,20	— 0,16°	+ 0,04°	— 0,20°	
51,40	— 0,21°	— 0,03°	— 0,18°	

$$[\alpha]_D^{20} =$$

$$+ 10^\circ \qquad + 50{,}02^\circ$$

$$[\alpha]_D^{20} = - 17{,}31^\circ$$

Spaltung von l-Leucyl-d-alanyl-glycin.

Stammlösung: 0,2120 g in 25 ccm physiologischer Kochsalzlösung (= 0,0327 Mol. im Liter).

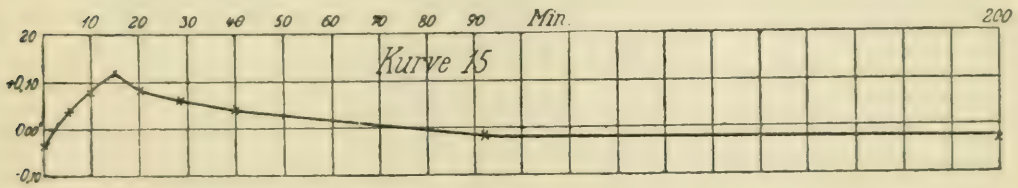
Mit Hefesaft B.

a) 5 ccm Stammlösung (= 0,0424 g) + 2,5 ccm Hefesaft B + 0,5 ccm physiologische Kochsalzlösung.

b) 2,5 ccm Hefesaft B + 5,5 ccm physiologische Kochsalzlösung.

b) konstant = — 0,02°.

a) Minuten	unkorr.	korr.
0	— 0,06°	— 0,04°
5	+ 0,02°	+ 0,04°
10	+ 0,06°	+ 0,08°
15	+ 0,10°	+ 0,12°
20	+ 0,06°	+ 0,08°
28	+ 0,04°	+ 0,06°
40	+ 0,02°	+ 0,04°
55	+ 0,06°	+ 0,08°
92	— 0,04°	— 0,02°
200	— 0,05°	— 0,03°

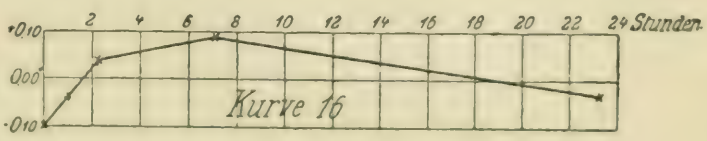


Mit Leberpreßsaft L.

a) 5 ccm Stammlösung (= 0,0424 g) + 1 ccm Saft L + 2 ccm physiologische Kochsalzlösung.

b) 1 ccm Saft L + 7 ccm physiologische Kochsalzlösung (konst. optisch inaktiv)

a) Stunden	korr.
0	— 0,10°
1	— 0,04°
2,20	+ 0,04°
2,66	+ 0,04°
5,10	+ 0,02°
7,10	+ 0,09°
23,10	— 0,03°

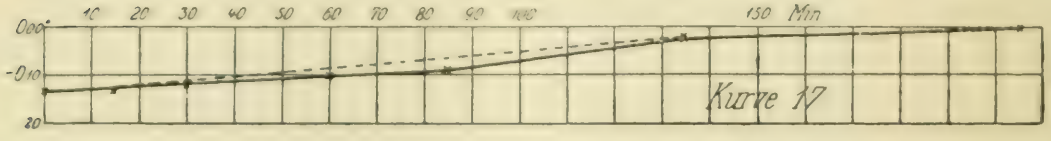


Mit aktiviertem Pankreassaft D.

a) 5 ccm Stammlösung + 2 ccm Saft D + 1 ccm physiologische Kochsalzlösung.

b) 2 ccm Saft D + 6 ccm physiologische Kochsalzlösung.

a) Minuten	unkorr.	korr.	b)
0	— 0,53°	— 0,13°	
15	— 0,53°	— 0,13°	
30	— 0,52°	— 0,12°	
60	— 0,50°	— 0,10°	= — 0,40°
85	— 0,49°	— 0,09°	
135	— 0,42°	— 0,02°	
203	— 0,36°	— 0,00°	— 0,36°
320	— 0,34°	— 0,00°	— 0,34°
340	— 0,28°	— 0,02° (konst.)	— 0,26°



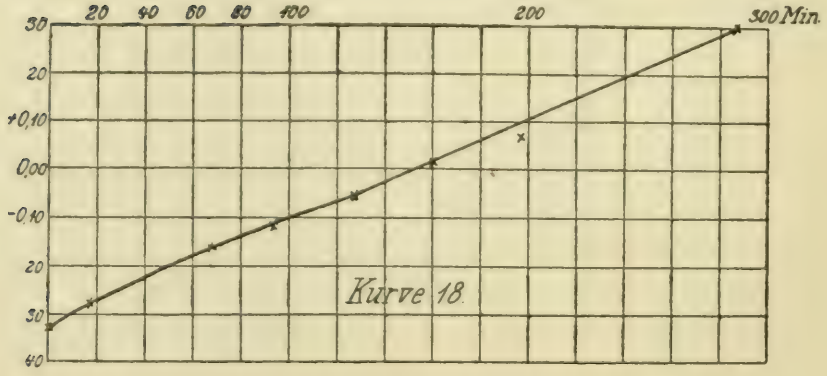
Spaltungen mit Pankreaspreßsäften.

I. Mit normalem Pankreaspreßsaft K′.

Spaltung von d-Alanyl-l-leucyl-glycin.

5 ccm der Stammlösung (= 0,1020 g) + 3 ccm physiologische Kochsalzlösung + 1 ccm Saft K′ (letzterer ist konst. optisch inaktiv).

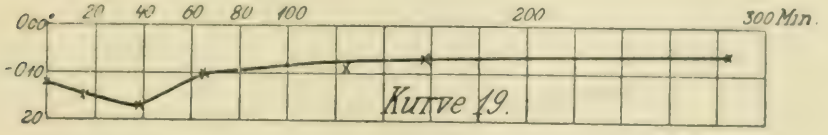
Minuten	
0	— 0,33°
17	— 0,28°
67	— 0,16°
93	— 0,12°
127	— 0,06°
160	+ 0,01°
197	+ 0,07°
287	+ 0,30°



Spaltung von d-Alanyl-glycyl-l-leucin.

5 ccm der Stammlösung (0,0995 g) + 3 ccm physiologische Kochsalzlösung + 1 ccm Saft K'.

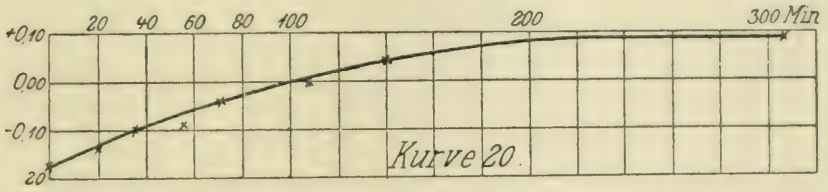
Minuten		Minuten	
0	— 0,13°	125	— 0,09°
15	— 0,15°	158	— 0,07°
38	— 0 17°	195	— 0,07°
65	— 0,10°	285	— 0,06°
92	— 0,08°		



Spaltung von l-Leucyl-d-alanyl-glycin.

8 ccm einer 1%igen Lösung des Tripeptids in physiolog. Kochsalzlösung + 1 ccm K'.

Minuten		Minuten	
0	— 0,17°	71	— 0,04°
20	— 0,14°	107	— 0,01°
35	— 0,10°	140	+ 0,04°
55	— 0,09°	305	+ 0,08°



II. Mit Pankreaspreßsaft K eines Diabetikers.

Spaltung von Glycyl-d-alanyl-l-leucin.

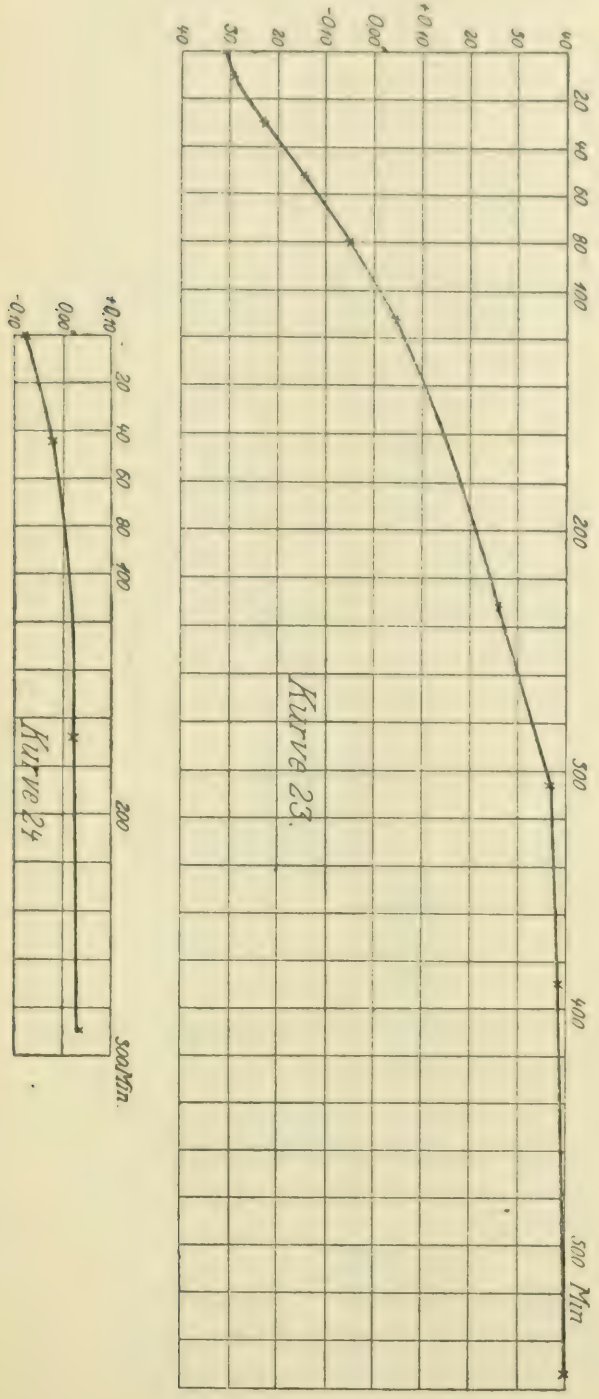
2,5 ccm Stammlösung (0,0569 g) + 5,5 ccm physiologische Kochsalzlösung + 1 ccm Saft K (letzterer ist konst. optisch inaktiv).

Minuten		Minuten	
0	— 0,47°	116	— 0,12°
20	— 0,34°	175	— 0,13°
35	— 0,27°	295	— 0,14°
55	— 0,14°	518	— 0,10°
73	— 0,15°		

Spaltung von d-Alanyl-glycyl-l-leucin.

5 ccm Stammlösung (0,0995 g) + 3 ccm physiologische Kochsalzlösung + 1 ccm K.

Minuten		Minuten		
0	— 0,14°	102	— 0,10°	
7	— 0,18°	222	— 0,10°	(Hierzu
17	— 0,13°	297	— 0,08°	Kurve 22.)
32	— 0,13°	442	— 0,07°	
70	— 0,10°			



Spaltung von
d-Alanyl-l-leucyl-glycin.

A. 5 ccm Stammlösung (= 0,1020 g)
+ 3 ccm physiol. Kochsalzlösung
+ 1 ccm K.

B. 2,5 ccm Stammlösung (= 0,0510 g)
+ 5 ccm physiol. Kochsalzlösung
+ 1 ccm K.

A. Minuten	
0	— 0,31°
11	— 0,29°
30	— 0,22°
53	— 0,14°
79	— 0,05°
111	+ 0,04°
231	+ 0,25°
306	+ 0,37°
390	+ 0,38°
453	+ 0,40°

(Hierzu Kurve 23.)

B. Minuten	
0	— 0,08°
8	— 0,02°
23	— 0,03°
44	— 0,03°
168	+ 0,02°
288	+ 0,03°
488	+ 0,00°

(Hierzu Kurve 24.)

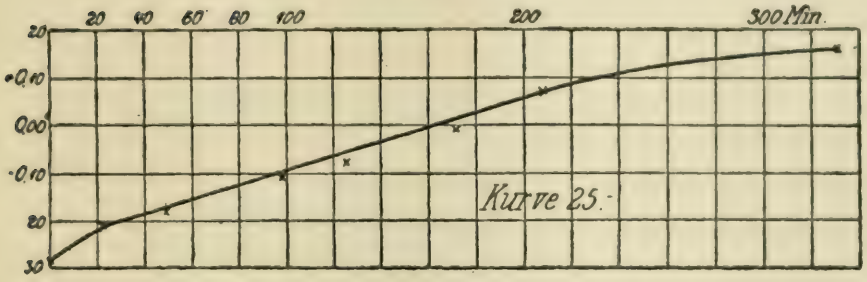
III. Mit Pankreaspreßsaft K″ eines Basedow-Patienten.

Spaltung von d-Alanyl-l-leucyl-glycin.

5 ccm Stammlösung (= 0,1020 g) + 3 ccm physiologische Kochsalzlösung + 1 ccm Saft K″ (dieser ist konst. optisch inaktiv).

Minuten	
0	— 0,29°
21	— 0,21°
49	— 0,18°
98	— 0,11°
125	— 0,08°
172	— 0,01°
207	+ 0,07°
332	+ 0,16°

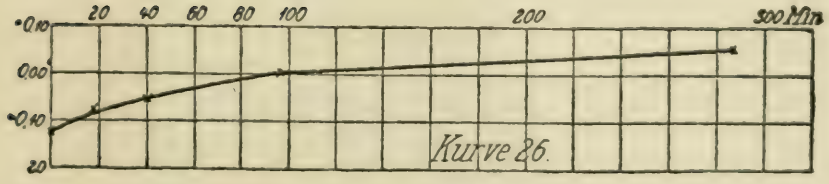
(Hierzu Kurve 25.)



Spaltung von l-Leucyl-d-alanyl-glycin.

8 ccm einer 1%igen Tripeptidlösung in physiolog. Kochsalzlösung + 1 ccm physiolog. Kochsalzlösung + 1 ccm Saft K″.

Minuten	
0	— 0,12°
17	— 0,08°
40	— 0,05°
195	— 0,00°
285	+ 0,05°



Einfluß von Natriumphosphat auf die Geschwindigkeit der Spaltungen.

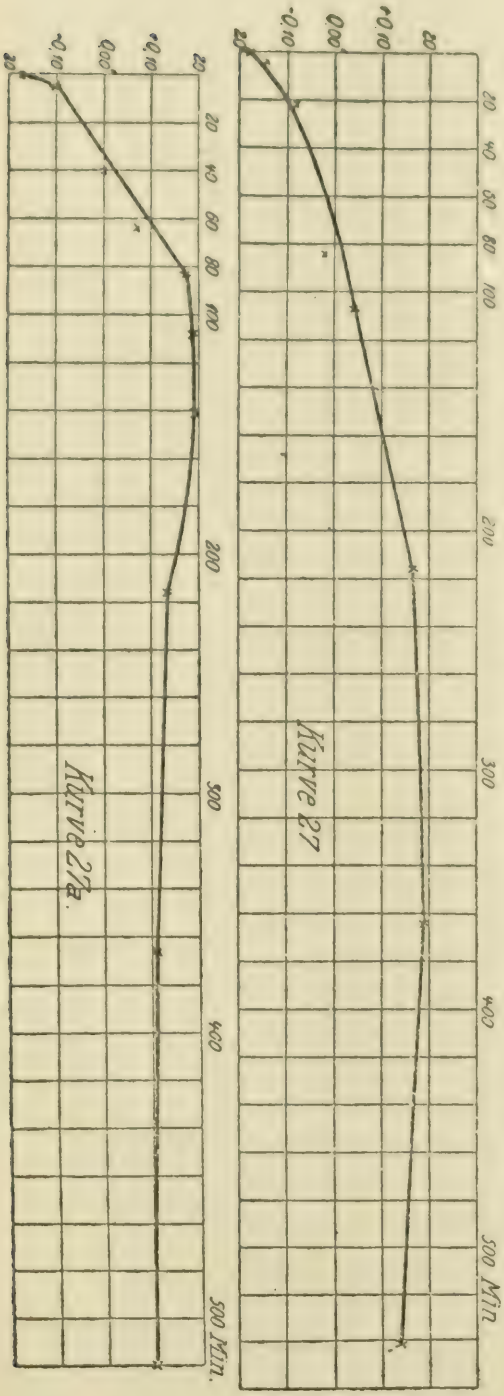
I. Hefesaft.

Spaltung von l-Leucyl-d-alanyl-glycin.

A. 8 ccm einer 1%igen Tripeptidlösung in physiologischer Kochsalzlösung + 1 ccm Saft B′.

B. 8 ccm einer 1%igen Lösung des Tripeptids in einer gegen Lackmus neutralisierten Lösung von physiologischer Kochsalzlösung + 2% NaH_2PO_4. Hierzu 1 ccm Saft B'.

Minuten	A.	B.
0	— 0,13°	— 0,14°
3	— 0,10°	— 0,10°
6	— 0,10°	— 0,08°
9	— 0,11°	— 0,10°
19	— 0,06°	— 0,06°
32	+ 0,01°	— 0,01°
73	+ 0,02°	— 0,00°



II. Leberpreßsaft.

Spaltung von l-Leucyl-d-alanyl-glycin.

A. Eine 1%ige Lösung des Tripeptids in physiologischer Kochsalzlösung wurde bereitet. 8 ccm hiervon + 1 ccm Leberpreßsaft L.

B. 8 ccm einer 1%igen Lösung des Tripeptids in einer gegen Lackmus neutralisierten Lösung von physiologischer Kochsalzlösung + 2% NaH_2PO_4. Hierzu 1 ccm Leberpreßsaft L.

C. 8 ccm der Phosphat-Kochsalzlösung + 1 ccm L. (Leberpreßsaft L ist optisch inaktiv.)

Min.	A.	Min.	B.	C.
0	— 0,17°	0	— 0,17°	
4	— 0,14°	4	— 0,10°	
22	— 0,08°	41	0,00°	
84	— 0,02°	64	+ 0,17°	
107	+ 0,04°	84	+ 0,18°	
141	+ 0,10°	107	+ 0,19°	α = 0,00°
216	+ 0,17°	141	+ 0,19°	
366	+ 0,19°	216	+ 0,14°	
541	+ 0,14°	366	+ 0,11°	
		541	+ 0,10°	

(Hierzu Kurven 27 u. 27a.)

III. Pankreaspreßsaft K′ (normal).

Spaltung von l-Leucyl-d-alanyl-glycin.

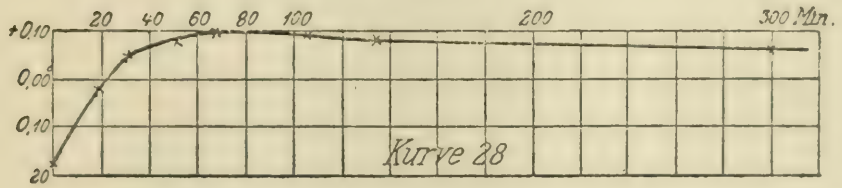
A. 8 ccm einer 1%igen Tripeptidlösung in physiologischer Kochsalzlösung + 1 ccm Saft K′.

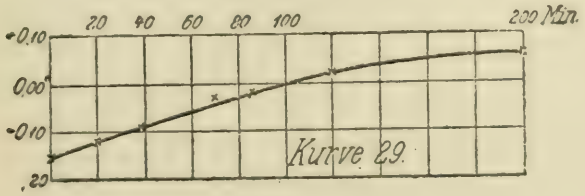
B. 8 ccm einer 1%igen Lösung des Tripeptids in einer gegen Lackmus neutralisierten Lösung von physiologischer Kochsalzlösung + 2% NaH_2PO_4. Hierzu 1 ccm Saft K′.

C. 8 ccm einer 1%igen Lösung des Tripeptids in einer gegen Lackmus neutralisierten Lösung von physiologischer Kochsalzlösung + 2‰ NaH_2PO_4.

A. Minuten		B. Minuten		C. Minuten	
0	−0,17°	0	−0,17°	0	−0,15°
20	−0,14°	17	−0,02°	20	−0,12°
35	−0,10°	30	+0,06°	38	−0,09°
55	−0,09°	50	+0,08°	70	−0,03°
71	−0,04°	67	+0,10°	85	−0,02°
107	−0,01°	105	+0,09°	120	+0,02°
140	+0,04°	135	+0,08°	200	+0,06°
305	+0,08°	300	+0,06°	Kurve 29.	
Kurve 20.		450	+0,03°		
		645	−0,05°		

Kurve 28.





IV. Pankreaspreßsaft K″ (Basedow).

A. Wie Lösung A sub III. Als Fermentsaft wurde 1 ccm Saft K″ + 2 ccm physiologische Kochsalzlösung hinzugefügt.

B. Wie Lösung B. sub III + 1 ccm Saft K″ + 1 ccm physiologische Kochsalzlösung.

Minuten	A.	B.
0	— 0,12°	— 0,12°
17	— 0,08°	— 0,05°
40	— 0,05°	— 0,01°
195	0,00°	+ 0,07°
285	+ 0,05°	+ 0,06°
	Kurve 26.	Kurve 30.

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